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1.0.0 Introduction

This handbook provides guidance to mission providers and Planetary Protection (PP) practitioners on implementing PP measures for both robotic and crewed space missions. This handbook represents major updates to PP practices since a previously drafted handbook from 2010. In 2017, NASA reorganized the Office of Planetary Protection (OPP) from the Science Mission Directorate (SMD) to a Technical Authority (TA) within the Office of Safety and Mission Assurance (OSMA). With this organizational change came a complete overhaul of NASA's PP policy and technical standards. This handbook reflects the latest NASA PP policy updates in ***NPR 8715.24***, *Planetary Protection Provisions for Robotic Extraterrestrial Missions* and technical requirements of ***NASA-STD-8719.27***, *Implementing Planetary Protection Requirements for Space Flight*. This handbook is a companion document to the NPR and technical standard and provides guidance, best practices, background information, and advice for practitioners to consider when implementing PP on NASA missions and NASA-partnered missions.

The guidance provided in this handbook covers a wide range of topics on the implementation of PP for different kinds of missions. Table 1 helps PP practitioners identify which parts of the document may be most applicable to their mission and role in the mission. *Chapter 2.0.0 PP Mission Categorization* details mission categorization.

Table 1: *Handbook Navigation by Mission Class*

	Cat I	Cat II	Cat III	Cat IV	Cat V	Laboratory	Analysis
Chapter 1	X	X	X	X	X		
Chapter 2	X	X	X	X	X		
Chapter 3		X	X	X			X
Chapter 4			X	X	X	X	
Chapter 5			X	X	X	X	
Chapter 6			X	X	X	X	
Chapter 7			X	X	X	X	
Chapter 8					X		
Appendix 1	X	X	X	X	X		
Appendix 2	X	X	X	X	X		
Appendix 3	X	X	X	X	X		
Appendix 4		X					
Appendix 5			X	X			X
Appendix 6			X	X			X
Appendix 7						X	

1.1.0 Background

PP has been a discipline of interest at NASA ever since the sterilization of space probes was first considered in 1959 to avoid planetary contamination during exploration.[1] The importance of PP grew with the focus of the first lunar landing missions, and NASA established the Planetary Quarantine Office in 1963.[2] The Planetary Quarantine Office would remain throughout the Apollo Missions until the focus changed to the broader term of "planetary protection" in the 1980s as NASA focused on planetary exploration.[3] A comprehensive history of PP at NASA is detailed in *NASA-SP-2011-4234, When Biospheres Collide: A History of NASA's Planetary Protection Programs*.[4]

The PP discipline is grounded in the Outer Space Treaty (OST) of 1967, which contains two key articles that form the foundation for PP. Article VI addresses the international responsibility that States Parties to the Treaty have for national activities in outer space, whether those activities are carried out by Governmental agencies or non-Governmental entities, and ensuring they conform with the provisions of the OST. For this reason, NASA's PP policy and technical standard apply to both NASA missions and NASA-partnered missions. Article IX of the OST focuses on controlling the risk of harmful contamination during exploration and research of outer space and avoiding adverse effects to the Earth from the introduction of extraterrestrial matter from other solar system bodies. It should be noted that the OST does not explicitly define "harmful contamination." The interpretation of "harmful contamination" changes as exploration technology evolves and scientific studies provide more knowledge. This flexibility is one reason why the OST has stood the test of time and remained relevant for more than 50 years.

The Committee on Space Research (COSPAR) was established in 1958 to coordinate worldwide space research. COSPAR is made up of an interdisciplinary scientific committee of national scientific organizations and international scientific unions and emphasizes the exchange of ideas, information, and opinions. The COSPAR Panel on Planetary Protection (PPP) forms international consensus guidelines for PP during solar system exploration based on current scientific knowledge. It also provides a forum for spacefaring nations without PP expertise to obtain advice on OST compliance. NASA supports and participates in the COSPAR process of developing new guidelines and provides input to the committee. However, COSPAR's focus is on the scientific exploration of space and purposefully does not define specific practices for PP implementation by missions, as that is the role of each of the States Parties to the OST. For this reason, NASA's PP policy and technical standard are informed by COSPAR policy, but additionally provide the detailed requirements for PP implementation by NASA missions and NASA-partnered missions to demonstrate compliance with the OST.

1.1.1 Spores and Planetary Protection

PP focuses on avoiding harmful biological contamination of solar system bodies during space exploration and preventing harmful biological contamination of Earth from missions returning extraterrestrial material. But how is that biological contamination quantified? On outgoing spacecraft, bacterial spores are used as an indicator of contamination. Bacteria represent a significant portion of life on Earth, and some bacteria form endospores, or "spores," to survive difficult environmental conditions. Spores are resistant to many environmental challenges, including temperature extremes, drying, radiation, and chemical exposures such as acids, oxidants, and other chemical disinfectants. Instead of dividing and replicating, a vegetative (active) cell sporulates and forms spores to remain viable when the surrounding environment is not ideal for growth. Once sporulation occurs, the spore can remain dormant for possibly tens of thousands of years before returning to a vegetative growth state.[5] PP practitioners use spore-forming bacteria as an indicator of biological contamination on flight hardware. The bacterial spore was selected as a biological indicator during the Viking Mars Program given its ability to survive the dry heat microbial reduction (DHMR) process and its status as the type of terrestrial organism that exhibited the highest potential to survive during the transit to and on Mars. Using the NASA Standard Assay (NSA), the presence of spores is measured by colony forming units (CFU)/m^2. Biological contamination requirements were developed for sensitive solar system bodies like Mars to avoid the risk of harmful contamination conducted by exploration activities.

[1]Meltzer, Michael. 2012. *When Biospheres Collide: A History of NASA's Planetary Protection Programs*. Government Printing Office.
[2]Ibid, 55.
[3]Rummel, John D. 2019. "From Planetary Quarantine to Planetary Protection: A NASA and International Story." Astrobiology 19 (4): 624–27.
[4]See footnote 1.
[5]Cano, R J, and M K Borucki. 1995. "Revival and Identification of Bacterial Spores in 25- to 40-Million-Year-Old Dominican Amber." *Science (New York, N.Y.)* 268 (5213): 1060–64.

The discipline of PP is also looking beyond spore-forming bacteria for modern and/or alternative ways to define and detect biological contamination. Modern technologies and processes such as metagenomics may provide deeper information about an organism's potential to survive in extreme environments.[6] This can be incorporated into target-body-specific decision making to improve upon how "harmful contamination" is defined for space exploration. These processes are still in development, but the goal is to offer additional tools for the PP practitioner community to support future scientific exploration of the solar system.

1.1.2 Major Handbook Principles

By updating the policy, technical standard, and handbook, OPP refines NASA's approach to implementing PP for spaceflight missions. The current approach includes increased flexibility and streamlining for reporting, gate products, and reviews; stronger alignment of PP implementation with existing NASA project management and systems engineering practices; and leveraging scientific consensus throughout the project lifecycle to adopt new practices and utilize the most current scientific information. The following sections further describe the major principles of the PP handbook: shifting emphasis from strictly prescriptive requirements to allow performance-based requirements and approaches. Various communication channels will increase communication between OPP and the PP practitioner community to enable utilization of this handbook as a living document by incorporating new information, approaches, and techniques as they evolve over time.

1.2.0 History of Bioburden Accounting

NASA policy on forward PP for Mars limits the number of spores on a spacecraft at launch (Category IV) to less than 5.0×10^5 total spores on all landed hardware (NASA-STD-8719.27 tables 4-3 and 4-4) with additional requirements depending on the subcategories (IVa, b, c).[7] Flight projects account for and reduce the number of spores (measured as CFU/surface area or CFU/volume) on the spacecraft prior to launch as required. This task can include rigorous cleaning procedures on the hardware, followed by culture-based bioassays to estimate the number of spores on spacecraft surfaces.

Spore requirements and methods have their origins in the Viking Mars Program, which was composed of two identical lander/orbiter combinations launched consecutively on August 20 and September 19, 1975. The focus of the Viking Project was to deliver life-detection experiments to the Martian surface, as well as other instruments that were designed to measure the Martian atmosphere, monitor its pressure and density, and observe and photograph the Martian surface. These experiments focused on obtaining scientific data relevant to the existence of extant life on the planet.

[6]Green, Stefan J, Tamas Torok, Jonathan E Allen, Emiley Eloe-Fadrosh, Scott A Jackson, Sunny C Jiang, Stuart S Levine, et al. 2023. "Metagenomic Methods for Addressing NASA's Planetary Protection Policy Requirements on Future Missions: A Workshop Report." Astrobiology 23 (8): 897–907. **https://doi.org/10.1089/ast.2022.0044**.
[7]**https://standards.nasa.gov/standard/NASA/NASA-STD-871927**

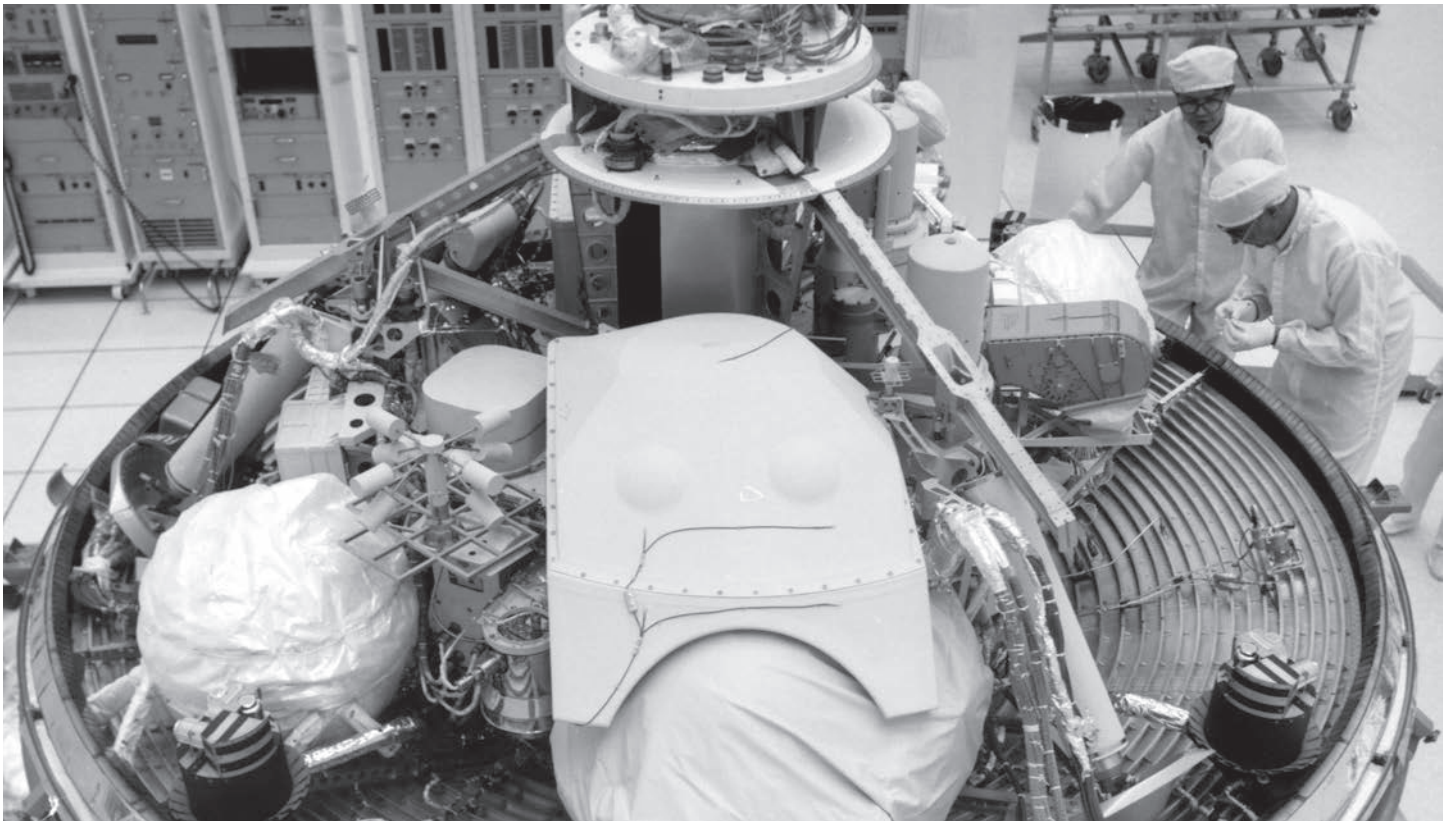

Figure 1: *At the Kennedy Space Center (KSC) in Florida, technicians inspect the Viking Lander 2 in Kennedy's Spacecraft Assembly and Encapsulation Facility 1. Photo credit: NASA*

For the Viking Mission, a complete viable microbial analysis and terminal sterilization (heat treatment) process was implemented to meet the PP probability of contamination requirements. Prior to launch, the Viking landers (VLC-1 and VLC-2) were evaluated for spore populations using the method that we now know as the NSA. This method was developed by the Viking team to support their initial analyses. The pre-bakeout spore distributions for Viking were used to support microbial reduction calculations associated with the formal heat treatment of each spacecraft.

Assays performed pre-sterilization on Viking-1 determined that, of a total of 1,000 ft^2 assayed, the spore burden density was 29.52 CFU/ft^2, or approximately 317 CFU/m^2.[8] The spore burden was 2.54 x 10^5 (surface), 1.47 x 10^3 (mated), and 1.55 x 10^5 (encapsulated). Viking-2 spore surface burden was 2.01 x 10^5 (see Table 2). These Viking cleanliness levels were used in development of the current NASA spore requirements. Levels of bacterial spores per square meter on the VLC-1 and VLC-2 were 1.6 x 10^2 and 9.7 x 10^1, respectively, prior to dry heat sterilization. To obtain the final bioburden on the spacecraft, the quantitative results were adjusted by a factor of two to compensate for the 50% efficiency factor of the swab-rinse technique.[9]

Table 2: *Viking Pre-sterilization Bioburden Data*[10]

Spacecraft	Average Spore Burden Density (CFU/m^2)	Spore Surface Burden (total spores)
Viking-1	317	2.54 x 10^5
Viking-2	Not available	2.01 x 10^5

[8]Corliss, William R. 1975. *The Viking Mission to Mars*, p. III-83.
[9]Puleo, J, N Fields, S Bergstrom, G Oxborrow, P Stebekis, and R Koukol. 1977. Review of Microbiological Profiles of the Viking Spacecraft. Applied and Environmental Microbiology 33 (2): 379–84.
[10]See footnote 8.

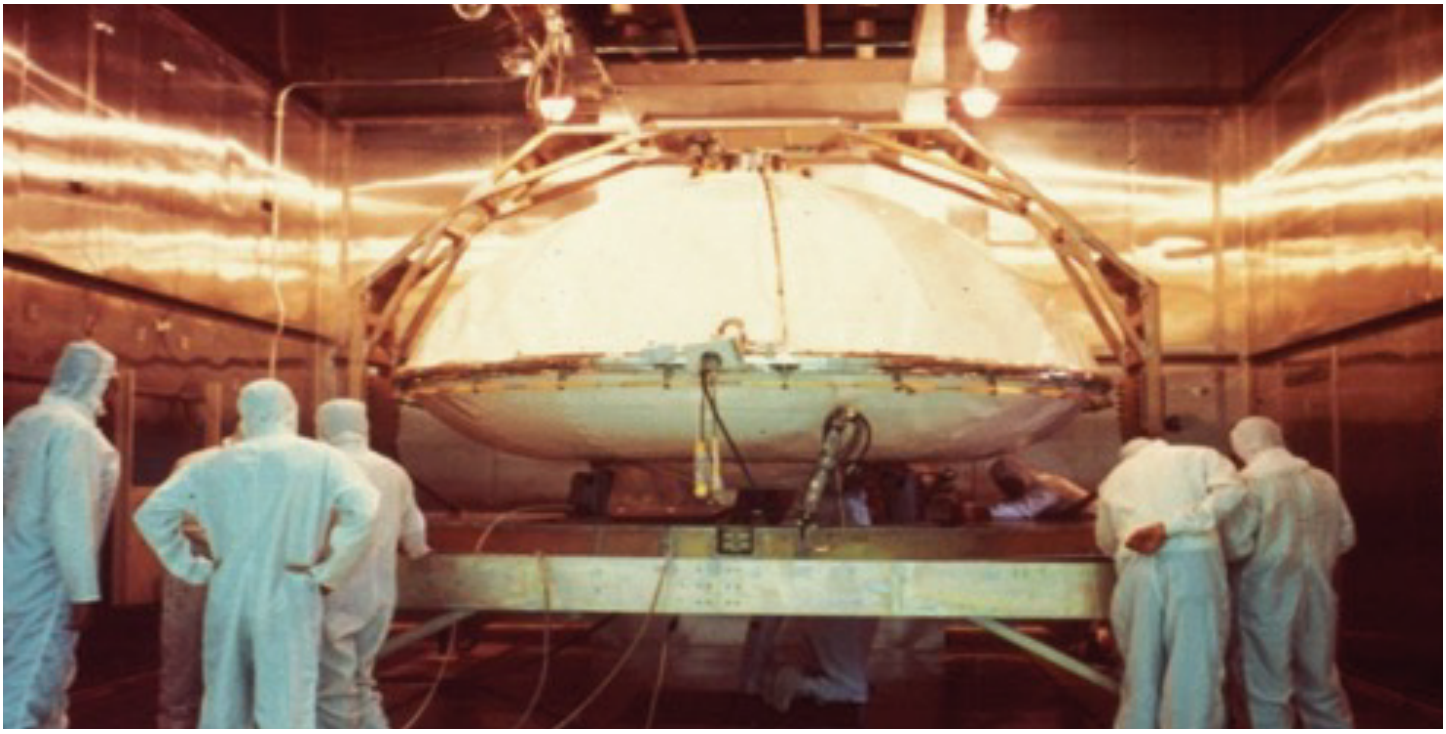

Figure 2: *Mars Viking landers were sterilized at KSC in ovens built specifically for the spacecraft. Photo credit: NASA*

Viking Program scientists developed the microbiological methods used to analyze Viking hardware surfaces. The resulting swab and wipe protocols consisted of wetting sample tools with sterile water and sampling the hardware to collect microorganisms on the sample tool surface. Once collected, the samples were transported to the microbiological laboratory for processing. Spore-forming bacteria were isolated with a heat-shock treatment, grown, and counted in the laboratory. This method is still used today and is known as the NSA.

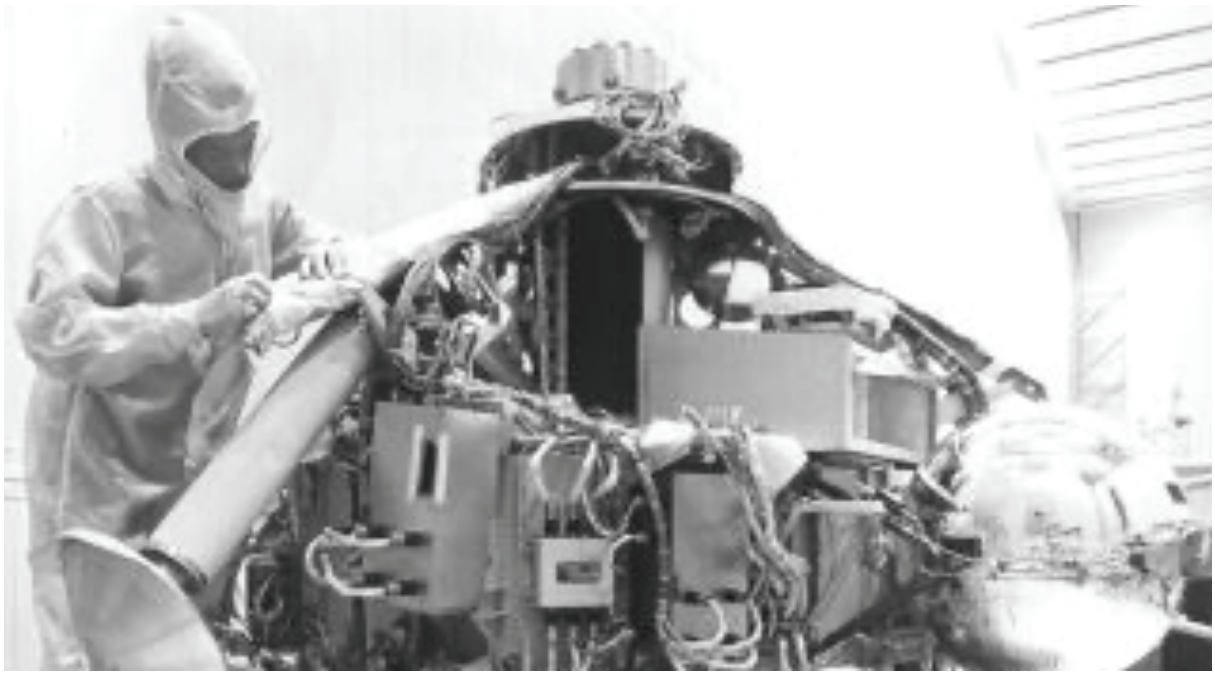

Figure 3: *Planetary protection samples were collected from the Mars Viking landers to verify biological cleanliness levels. Photo credit: NASA*

1.3.0 Adoption of New Standards and Approaches

A key change to NASA-STD-8719.27 compared to its predecessor is the addition of performance-based requirements and approaches to PP. The performance-based approach communicates the desired "to-be" state of a system and allows for engineering trades and innovative processes to be utilized to reach that state. It communicates the desired end state of the product, rather than detailing the exact steps and processes to be performed to reach the end state. Conversely, prescriptive requirements and approaches explicitly state what is to be done and how to do it. Prescriptive requirements and approaches are useful in cases where definite steps or conformance to a process are important. For example, the NSA detailed in NASA-STD-8719.27 is an example of a prescriptive process for performing spore assays to determine spacecraft bioburden levels. An assurance case is an approach to meeting a performance-based requirement.

The updated technical standard expanded the ability for mission providers to utilize prescriptive approaches, performance-based approaches, or a combination of both. PP must be flexible as NASA considers challenging missions like crewed missions to Mars. The existing prescriptive robotic requirements of a static bioburden density is not implementable with the added complexity of dynamic microbial sources, so alternative approaches are needed to prevent harmful contamination. Performance-based approaches increase the number of options available to meet PP requirements when considering unknowns or ill-defined systems. A high level of knowledge about a system is needed to define prescriptive requirements.

The use of international standards and alternative approaches is also incorporated into NASA's PP policy and standard. Mission providers may choose NASA standards and processes or international standard processes when implementing PP. OPP reviews the appropriate use of these standards to ensure alignment with NASA's PP policy. For example, a European standard for dry heat bioburden reduction of flight hardware may be utilized by a mission provider.[11] The mission provider can benefit by using an existing international standard, and NASA benefits by not needing to expend resources to develop a duplicative standard. A mission provider may also wish to implement a new method they developed for meeting PP requirements. Mission providers may propose these methods as alternatives to the standard methods. OPP reviews these methods prior to adoption, using data and information provided by the proposer of the method. Alternative methods are rigorously reviewed, and OPP may draw from current scientific consensus, call upon subject matter experts (SMEs) for further review, or request additional analysis and information prior to the alternative method being deemed acceptable.

1.3.1 Communication Mechanisms

This handbook provides supporting information to help projects, missions, and PP practitioners implement best practices and meet the requirements in the NPR and technical standard but is not the only communication mechanism between the OPP and the PP practitioner community. The OPP has developed training materials for internal NASA use to be accessed from the System for Administration, Training, and Educational Resources for NASA (SATERN) learning management system. Additionally, OPP has developed a series of informational articles and short videos and made them publicly available on NASA's Web site.[12] OPP also participates in town hall events, national and international conferences, and site visits to speak about the latest in PP research, technology development, and status of NASA missions with PP implications, and to answer questions from the PP practitioner community. PP practitioners are encouraged to contact OPP with any questions related to the NPR, technical standard, handbook, or other PP-related topics.[13]

1.3.2 A Living Document

Approaches and implementation practices to satisfy PP requirements, and the requirements themselves, will continue to change as NASA explores new areas of the solar system with robotic and crewed missions. This handbook is a living document and will continue to be updated over time with the latest information. Comments on information to be updated or included in future handbook revisions may be submitted to the OPP through an online Web form at **https://sma.nasa.gov/sma-disciplines/planetary-protection**.

[11] **https://ecss.nl/standard/ecss-q-st-70-57c-dry-heat-bioburden-reduction-for-flight-hardware-30-august-2013/**
[12] **https://sma.nasa.gov/sma-disciplines/planetary-protection/explore**
[13] **https://sma.nasa.gov/sma-disciplines/planetary-protection**

2.0.0 PP Mission Categorization

Navigation Summary: *Chapter 2 discusses the process for defining the PP categories for NASA or NASA-supported missions. This chapter is applicable to all mission types during mission design and formulation.*

The practice of PP focuses on two main phases of space exploration. The first is to control the risk of harmful contamination to bodies in the solar system other than the Earth during space exploration activities and to protect the integrity of the search for and study of processes of chemical evolution or origin of life. This is known as forward PP. The second, known as backward PP, focuses on preventing potentially harmful consequences for humans and the Earth's environment due to the return of extraterrestrial samples to Earth. Not all solar system bodies have the same level of sensitivity to contamination during forward PP, nor the same potential risk to humans and Earth's environment during backward PP. Missions with PP implications are divided into separate categories to account for this range of sensitivity and potential risk.

PP mission categorization defines a mission's biological and relevant molecular contamination risk in a process that considers the target solar system body to be explored, additional target bodies that may be encountered during the mission, and mission hardware and operations during exploration. Based on this contamination risk, a range of technical requirements are then implemented and documented to control and manage the risk to an acceptable level. The practice of categorization ensures the international space exploration community controls contamination of solar system bodies to levels agreed upon by the international scientific community. This also allows for technical requirements and implementation practices to continuously evolve within each PP mission category. Each subsequent mission benefits from the scientific knowledge gained and lessons learned from previous missions to the same target solar system body or category of bodies. NASA's OPP maintains a listing of all prior missions, their categories, and reference reports on a publicly accessible OSMA **Web site**.

2.0.1 Overview of the Categories

The COSPAR PPP maintains their PP policy for the reference of spacefaring nations to guide compliance with the OST. This policy is based upon the most current, peer-reviewed scientific knowledge to enable the exploration of the solar system. Within this policy, COSPAR defines five top-level PP mission categories: Categories I – IV relate to forward PP and Category V relates to backward PP. NASA participates in the COSPAR PPP policy development process, is guided by the COSPAR policy and guidelines, and incorporates the PP mission categories into NPR 8715.24 and NASA-STD-8719.27.

PP mission categories consider two main inputs as shown in Figure 4: target body and mission type, both of which consider the likelihood of causing harmful contamination. Target bodies are classified based on whether there is scientific interest in the study of chemical evolution and the origin of life and whether contamination at that target could interfere with future investigations. For the significance of contamination, each category considers: 1) the criticality of the mission's scientific investigation in understanding the process of chemical evolution or origin of life, and 2) the chance that contamination from the exploration activities could compromise future investigations.

Mission types are ranked by increasing probability of contamination. At the lower end of risk of contamination are missions conducting flybys or orbiting a target body. Missions that have direct contact with the target body as part of the primary mission objective such as probes and landers have an increased risk for contaminating the target body. Finally, Earth return missions are the last mission type, which are further divided into unrestricted Earth return and restricted Earth return based on the classification of the target body from which they are returning to Earth. Unrestricted Earth return missions have a very low risk of contaminating Earth when returning material from the explored target body because the scientific community does not think life ever existed on these bodies. Restricted Earth return missions are required to implement high containment controls to ensure that returned material is not released before sterilization or sample safety assessment.

Together, the target body and mission type are inputs into the process of assigning a PP mission category. As shown in Table 3, Category I missions include all mission types targeting bodies that are not of direct interest for understanding the process of chemical evolution or where exploration would not be compromised by terrestrial contamination. The significance of

contamination slightly increases for Category II missions which do have significant interest relative to the process of chemical evolution, but with only a remote chance that contamination would compromise current or future investigations. Category III missions are for missions with no direct contact with the target body (flyby, orbiter, or gravity assists) but the target body is of significant interest relative to the process of chemical evolution and/or the origin of life and, and the body has a significant risk that contamination could compromise future investigations. Category IV targets have the same heightened sensitivity to contamination as Category III targets, but Category IV missions have direct contact with the target body, such as probes and landers.

Earth return missions receive both a forward and a backward PP mission categorization. The forward categorization protects the target body to be explored, while the backward categorization is based on the risk that exploration activities would cause adverse changes in the environment of the Earth-Moon system from returning material from the target body. Category V unrestricted Earth return, or Category V(u), is determined based on scientific evidence that the mission will not cause adverse changes in the environment of the Earth-Moon system and has no additional PP requirements.[14] Category V restricted Earth return, or Category V(r), is used if scientific evidence does not support the Category V(u) categorization.

The outputs of the mission categorization process, as shown in Figure 4, are implementation guidelines and practices to control contamination and recommended reporting practices. The level of implementation needed to control contamination to an acceptable level and the level of reporting all depend on the specific scientific investigation of the mission, the target body to be explored, and the operations of the mission during exploration. Categorization provides a means to track and understand how each mission is exploring the solar system with respect to contamination risk and conducting activities so as not to introduce adverse changes in the environment of the Earth-Moon system.

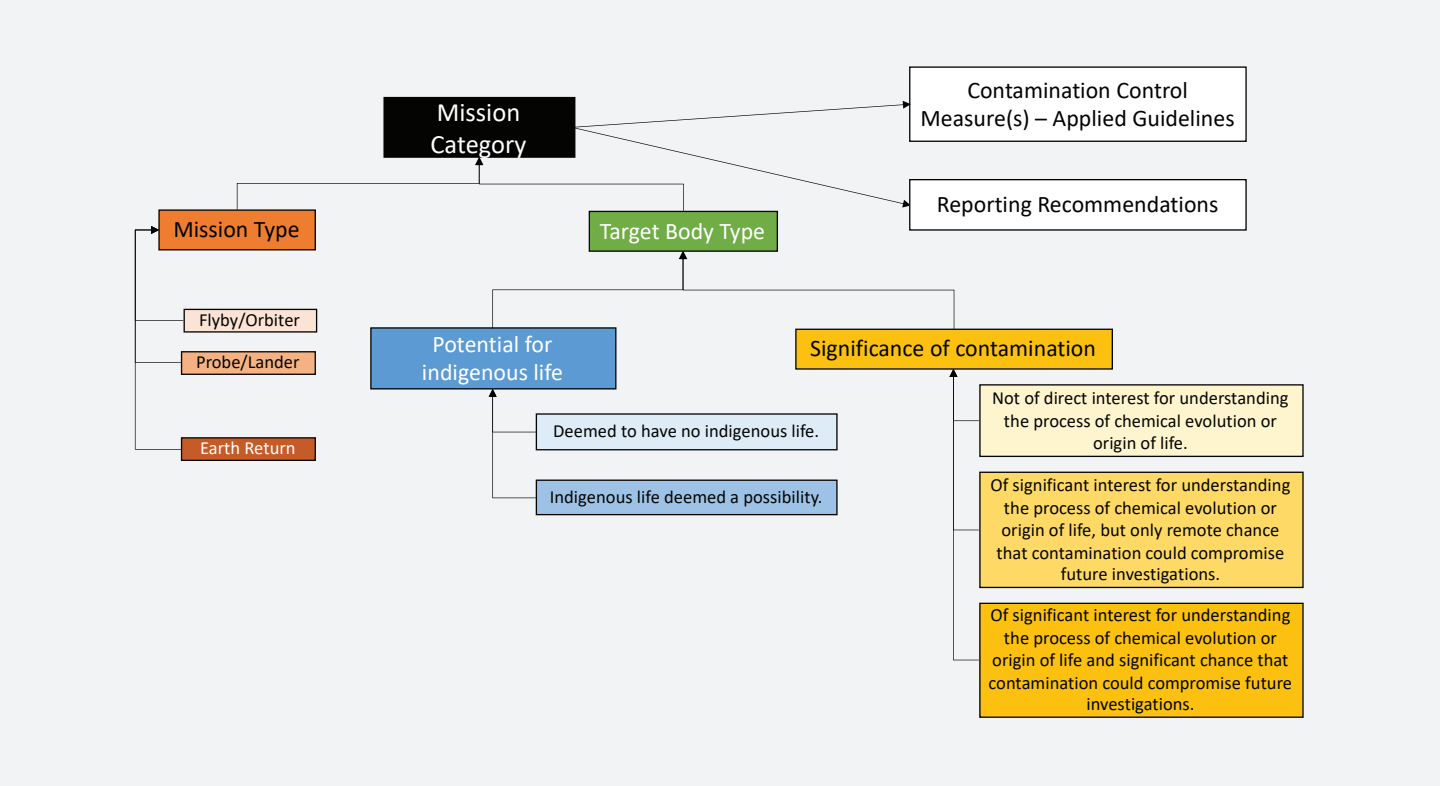

Figure 4: *Inputs and Outputs in PP Mission Categorization*

[14] https://nap.nationalacademies.org/catalog/6281/evaluating-the-biological-potential-in-samples-returned-from-planetary-satellites-and-small-s olar-system-bodies

Table 3: *PP Mission Categorization Summary*

		Planetary Protection Mission Category	Mission Type	Target Body Type: Potential for Indigenous Life	Target Body Type: Significance of Contamination: Understanding the process of chemical evolution or origin of life	Target Body Type: Significance of Contamination: Chance that contamination could compromise future investigations
Increasing Biological Contamination Risk ↓	Forward Planetary Protection	Category I	All outbound types	None	Not of direct interest	None
		Category II	All outbound types	None	Significant	Remote
		Category III	No direct contact: Flyby, orbiter, gravity assist	Possible	Significant	Significant
		Category IV	Direct contact: Lander/probe	Possible	Significant	Significant
	Backward Planetary Protection	Category V(r) - Restricted	Earth return	Possible	Significant	Significant
		Category V(u) - Unrestricted	Earth return	None	--	--

PP categorization drives which types of implementation strategies may be used to meet PP requirements. Table 4 provides an overview of the types of PP implementation guidelines for selected target bodies. Note that this is not a comprehensive list of all target bodies for which PP applies but a representative example of common target bodies. The detailed mission documentation expected elements can be found in the appendices of NASA-STD-8719.27. Cleanroom operations and biological cleanroom operations are further detailed in *Chapter 5.0.0 Biologically Controlled Cleanrooms*. Inadvertent impact and trajectory biasing mission design and navigation parameters are covered in *Sections 3.3.0 Inadvertent Impact at Mars for Lunar (Earth-Moon System) Missions* and *3.4.0 Generalized 50-Year Probability of Impact Requirement Compliance for Mars Missions*, respectively. Organic inventory and archiving overview can be found in *Chapter 7.0.0 Biological Estimation Techniques. Chapter 6.0.0 NASA Standard Assay Laboratory Considerations* and *Section 7.3.0 Bioburden Accounting Parameters* cover the biological control assessment and accounting parameters that a mission may need to assess biological contamination on a spacecraft and spacecraft-associated surfaces.

Table 4: *Examples of PP Implementation Guidelines for Commonly Explored Target Bodies*

Guidelines						
Category	**Mission Documentation**	**Cleanroom**	**Trajectory Biasing**	**Inadvertent Impact**	**Organic Inventory**	**Biological Control**
Earth's Moon	Yes	-	Yes	-	Yes	-
Titan	Yes	Yes	-	-	Yes	-
Jupiter	Yes	Yes	-	-	Yes	-
Venus	Yes	Yes	-	-	Yes	-
Mars	Yes	Yes	Yes	Yes	Yes	Yes
Europa	Yes	Yes	Yes	Yes	Yes	Yes
Enceladus	Yes	Yes	Yes	Yes	Yes	Yes

2.0.2 Factors to Consider for Categorization

In the simplest form, PP categorization considers these questions:

1. Where is the mission going?
2. How is it getting there?
3. What is it going to do when it gets there?

Expanding upon these questions further, the following factors provided in Table 5 should be considered in the process of determining PP mission categorization.

Table 5: *Factors To Consider for PP Mission Categorization*

Factor #1	What is the target body to be explored?	The destination target body to be explored is usually the first factor to consider, and the table of Appendix C of NPR 8715.24 provides example solar system target bodies and categorization.
Factor #2	What are the main characteristics of the trajectory, including flybys?	Different solar system bodies have different sensitivities to contamination, and the potential for all launched hardware to impact any solar system body (the target of the mission or other body) must be considered. This includes all propulsion stages.
Factor #3	What is the mission architecture at the target body?	Will the mission involve hardware that will be orbiting, landing, probing, or exploring on the surface, in the atmosphere, or beneath the surface? Does the mission architecture have multi-element missions? Mission operations must be considered for all hardware as each operation has a different potential for contamination of the target body.
Factor #4	What is the instrument payload?	What science investigations will the instrument payload conduct? Will the instrumentation be performing life detection investigations or exploring special regions? Are secondary or auxiliary payloads included? Missions are now including ride-along payloads that may be deployed en route to the target body or separately from the primary payload at the target body. The potential for each payload to contaminate the target body or any other solar system body encountered along the way must also be considered.
Factor #5	What is the end-of-mission plan for all hardware?	When the hardware reaches its end of life, will it shut down in place or transfer to a different location? All locations that could result from an unsuccessful disposal maneuver (such as de-orbiting) or relocation on the surface by natural processes such as wind forces or seasonal freezing and thawing should be considered as well.

The information obtained from considering these five factors together typically provides enough information to assign the mission a PP category. In the process of PP mission categorization, there may be several different solar system bodies in which the mission hardware will potentially interact. It is important to note that the most sensitive solar system body will drive the PP mission categorization. For example, a mission targeting an asteroid (a Category I target body) will perform a flyby of Mars (a Category III target body). Therefore, the mission would be assigned a PP Category III mission due to the Mars flyby.

PP mission categorization sets into motion several subsequent steps, including requirements definitions, analyses, mission design efforts, and PP implementation processes. The resources required to plan, track, implement, and verify PP requirements on a mission can vary greatly between missions assigned to different PP categories. This is why it is important to complete the process of PP mission categorization by the System Requirements Review (SRR) as defined in NPR 8715.24.

2.0.3 Key Roles in the Categorization Process

Under previous versions of the NASA PP policy, missions would request and receive mission PP categorization assignments through a letter from the OPP. NPR 8715.24 defines a new process for categorization with expanded roles participating in the PP mission categorization process as shown in Figure 5. The process now involves key members of the programmatic authority as well as members of the SMA TA.

The project-level SMA TA supports the relationship between the project and the OPP and seeks to engage this relationship as early as possible in the project lifecycle. The SMA TA advises projects to notify the OPP of missions requiring PP mission categorization as early as proposal development and architecture formulation. As a response, the NASA Project Manager notifies the OPP of missions requiring PP mission categorization to begin a dialogue and consultation with the OPP. Once the NASA Project Manager has drafted a categorization request with the proposed mission categorization, the categorization request is sent to the Mission Directorate Associate Administrator (MDAA). The MDAA coordinates with the OPP in reviewing the categorization request. For PP Category I and II missions, the OPP provides concurrence for the SMA TA. For PP Category III, IV, and V missions, the OPP provides recommendations for concurrence to the Chief of SMA, who then provides concurrence to the MDAA. The MDAA provides the final PP mission categorization assignment to the NASA Project Manager.

This process may seem to introduce several steps, whereas the previous process only had one step (the OPP providing a categorization letter to the project). However, this process now creates a system of checks and balances to ensure the appropriate programmatic and SMA TA authorities are involved in the process of PP mission categorization. This also ensures the appropriate authorities have an ownership role in NASA's PP processes and that the OPP maintains technical oversight of the process.

Additionally, other specific roles [e.g., PP engineer, Contamination Control (CC) engineer, SMA discipline lead, etc.] for the mission or project may contribute to the categorization process, but these are ultimately responsible to the NASA Project Manager or SMA TA for determination of the category as described in NPR 8715.24.

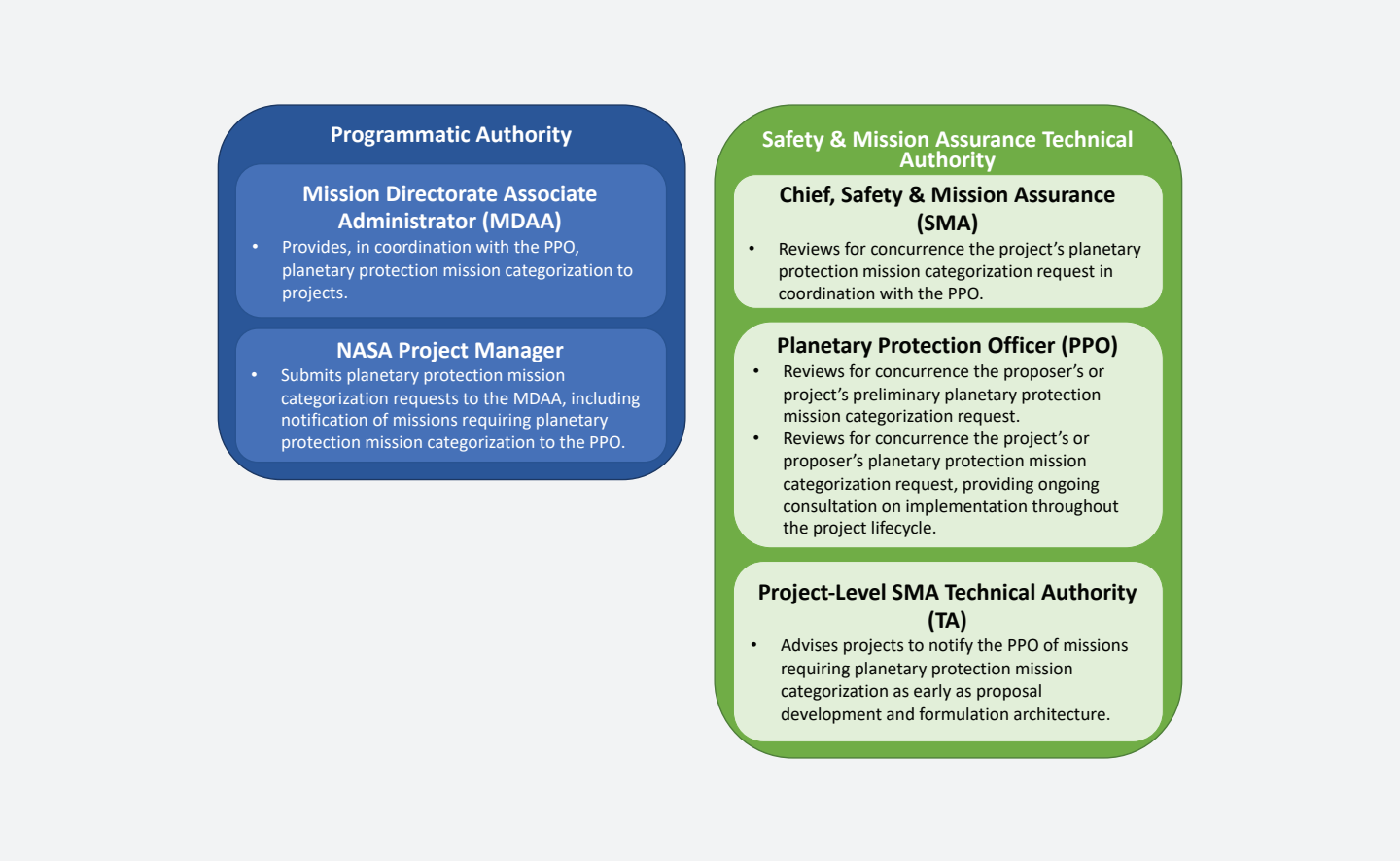

Figure 5: *Key Roles in the PP Mission Categorization Process*

2.0.4 Updates and Changes To PP Mission Categories

The PP mission categories may be updated or changed based on scientific consensus resulting from new scientific knowledge. As missions explore the solar system and gain new information, this information can provide new scientific insight, which must be looped back into the international PP community to ensure the community is following the latest practices. The COSPAR PPP frequently engages with the planetary science and PP communities to review the latest scientific knowledge gained and consider any impacts to PP mission categories. When an update is warranted, COSPAR issues a new PP policy incorporating any changes or updates. NASA participates in the COSPAR PPP and provides input into PP policy considerations. Additionally, NASA may consult with the National Academies for Science, Engineering, and Medicine (NASEM) for key scientific insight and then provide the NASEM study results and recommendations to the COSPAR PPP for policy recommendations. NASA also maintains NPR 8715.24 and NASA-STD-8719.27 and updates these documents with any key changes in PP mission categorization resulting in a change in scientific consensus. The needs of the scientific exploration community drive both COSPAR and NASA PP policies and the defining characteristics of PP mission categories.

2.1.0 Subcategories

There are several instances in which the initial I-V categorization gets further refined based on target body and mission operations. These include missions to Earth's Moon, Category II* bodies, missions landing on Mars, and sample return missions.

2.1.1 Missions To Earth's Moon

Missions to Earth's Moon are typically categorized as PP Category II. In 2021, COSPAR PPP considered questions raised by the scientific community about future exploration of Earth's Moon, and in particular, the science concerning chemical evolution and the interest in volatile molecules potentially trapped in the lunar poles and permanently shadowed regions (PSRs). The PPP agreed to update the categorization and requirements for missions to Earth's Moon by maintaining the Category II designation, but with two additional subcategories.[15] Category IIa includes all missions to the surface of the Moon whose nominal mission profile does not access areas defined in Category IIb. The material inventory required for Category IIa is limited to organic products that may be released into the lunar environment by the spacecraft propulsion system. Category IIb includes all missions to the surface of the Moon whose nominal mission profile accesses PSRs and the lunar poles, in particular latitudes south of 79 °S and north of 86 °N. A Category IIb mission requires a full organic inventory, including solids and volatiles. Table 6 summarizes these designations and requirements for Category IIa and IIb.

Table 6: *Subcategories for Missions To Earth's Moon*

Category	Descriptions	Volatiles Released by Propulsion System	Spacecraft Organic Inventory
II	Orbiter	—	—
IIa	Lander, Moon surface not within IIb	Required	—
IIb	Lander, PSRs, and lunar poles	Required	Required

2.1.2 Category II* Target Bodies

The Category II* (pronounced "two star") target bodies defined in NPR 8715.24 can create confusion in the process of PP mission categorization. The Category II* target bodies are icy satellites where there is a remote potential for contamination of the liquid-water environments, such as Ganymede (Jupiter); Titan (Saturn); or Triton, Pluto, and Charon (Neptune). The most important point to remember to alleviate some of the confusion is that **Category II* only describes target bodies, and there are no Category II* missions.**[16] When a mission plans to explore a Category II* target body, this should trigger a PAUSE in the categorization process. In this **pause**, the mission design and most current scientific consensus are evaluated for the potential to contaminate the target body based on the body's ice thickness, presence and abundance of aqueous environments, and plausible contamination pathways at those locations that could lead to a biological inoculation event. If analysis demonstrates that there is a remote potential for contamination of the target body as described in Section 4.2.2.2 of NASA-STD-8719.27, then the mission is designated a PP Category II mission. If a remote potential for contamination is not demonstrated, or the analysis is not performed, then the mission is designated a PP Category III mission for orbiters, flybys, and gravity assists, or PP Category IV for landers. Figure 6 illustrates this decision-making process for evaluating missions targeting Category II* target bodies.

[15]COSPAR Business, Space Research Today, Volume 211, August 2021, Pages 9-11. **https://www.sciencedirect.com/science/article/pii/S1752929821000360?via%3Dihub** (Click the link to the PDF at the top.)
[16]See Note 3 of Appendix C of NPR 8715.24.

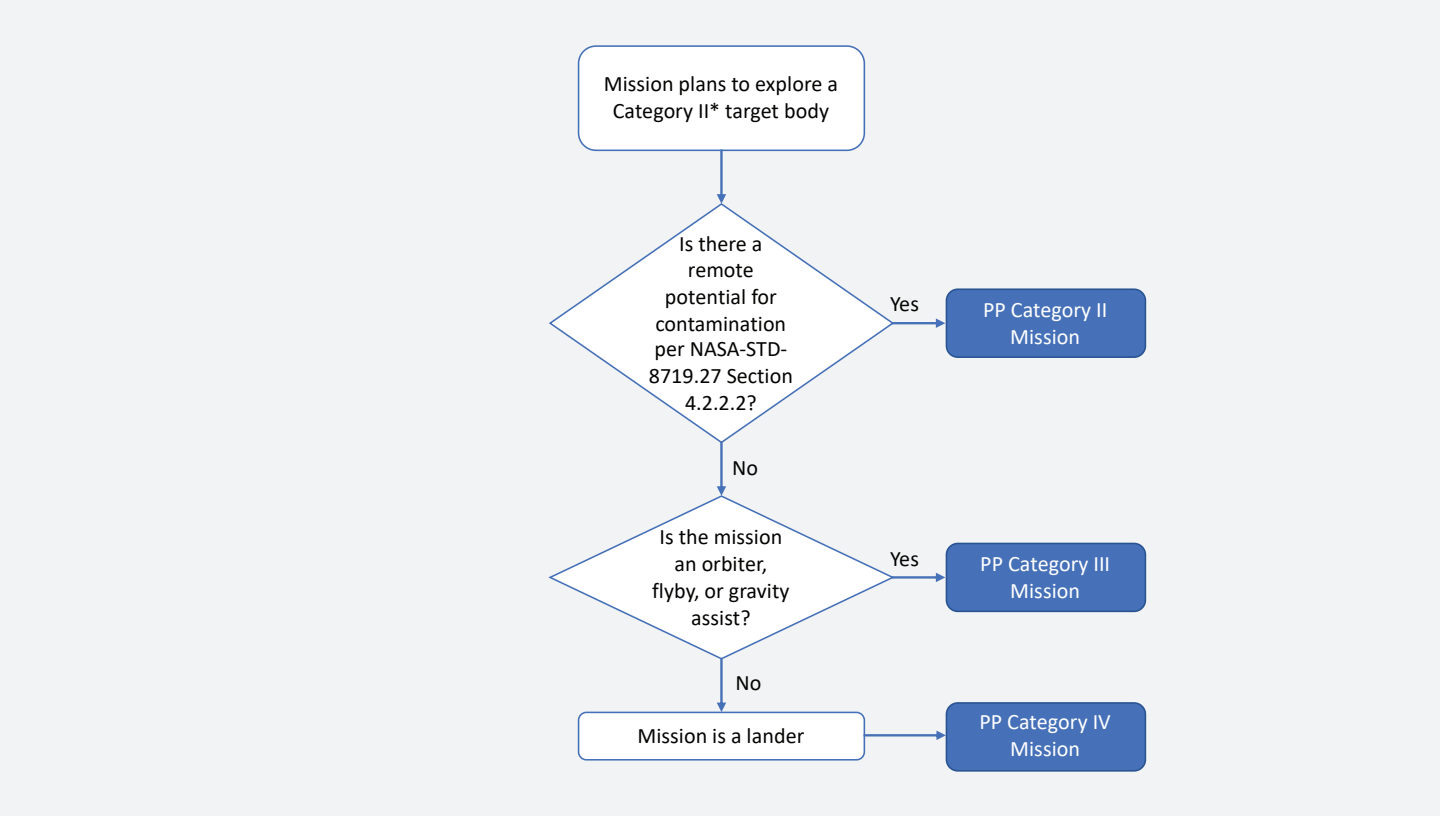

Figure 6: *Decision Process for Evaluating Category II* Target Bodies*

2.1.3 Landed Missions on Mars

Missions landing on Mars have a forward PP classification of Category IV, and this categorization is further divided into three subcategories depending on if the mission has a goal for detecting life on Mars, returning samples from Mars, or if the mission will access a special region as shown in Table 7. A special region is a location on Mars where temperature and water activity conditions are favorable for terrestrial microorganisms to potentially replicate. Special regions are discussed in more detail in *Section 2.1.4 Special Regions (Mars)*. Sample return missions also have a backward PP classification, which is discussed in *Section 2.1.5 Sample Return Missions*. If a mission to Mars has no goal for detecting life on Mars or will not be returning samples and will not be accessing a special region, then the mission is categorized as Category IVa. A mission with a life detection goal or returning samples but not accessing special regions is categorized as Category IVb. A mission accessing a special region is categorized as IVc. If a mission has both a life detection goal or sample return and is planning to access special regions, it is categorized as both IVb and IVc. These missions are evaluated on a mission-by-mission basis and combine the requirements of both Category IVb and IVc. All requirements are detailed in NASA-STD-8719.27.

Table 7: *Subcategories for Landed Missions on Mars*

Forward PP Category	Life Detection or Sample Return	Accessing a Special Region
IVa	No	No
IVb	Yes	No
IVc	No	Yes
IVb & IVc	Yes	Yes

2.1.4 Special Regions (Mars)

At the end of the Viking era of exploration, Mars was considered to be a cold, dry desert, bathed in solar UV light and deemed inhospitable to life. However, by the late 1990s, missions such as the Mars Global Surveyor orbiter and the Mars Pathfinder lander had shown Mars to be a diverse place of fluvial processes with evidence of ancient, long-lived bodies of past surface water. These findings culminated in the recognition that some locations on Mars are more important from an astrobiologically relevant point of view than others. Given that these locations on Mars are habitable for viable terrestrial microorganisms, they are more vulnerable to forward contamination, and thus a higher degree of protection is warranted than the general Mars environment. As a result, the concept of special regions was included in the 2002 COSPAR PP policy.[17]

A special region is a location on Mars within which terrestrial microorganisms are likely to replicate [i.e., where the environmental parameters in terms of water activity and temperature thresholds (that must be satisfied at the same time, for such replication to occur) are a water activity of > 0.5 and a temperature of > 28 °C]. Recommendation 13 of the NRC 2006 Preventing the Forward Contamination of Mars (PREVCOM) report stated, "Until measurements are made that permit distinguishing confidently between regions that are special on Mars and those that are not, NASA should treat all direct-contact missions (i.e., all Category IV missions) as being in [current Category IVc]."[18]

The position of the PREVCOM committee, if implemented, would have been onerous for future Mars missions, so NASA sought, through the Mars Exploration Program Analysis Group (MEPAG), to obtain a better way to define special regions based on available data. The MEPAG Special Regions – Science Analysis Group (SR-SAG), by defining the habitable environment of the Martian surface and shallow subsurface in terms of two known/knowable parameters (water activity and temperature), fulfilled the PREVCOM requirement to distinguish "special" from "not special," identified geographic features on Mars that might be considered "special," and concluded that the majority of modern Mars is "not special."[19] These U.S. findings were endorsed by the international community at a COSPAR symposium[20] and updated to the present situation in 2014.[21]

Category IVc robotic Mars missions are required to either restrict the entire landed system to a surface bioburden level of ≤ 30 spores, OR sterilize the subsystems that directly contact the special region to these levels (and a method is provided for preventing their recontamination prior to accessing the special region). If a non-nominal condition (such as a hard landing) could cause a high probability of inadvertent biological contamination of the special region by the spacecraft, the entire landed system must be sterilized to a surface bioburden level of ≤ 30 spores and a total (surface, mated, and encapsulated) bioburden level of ≤ 30 + (2 x 10^5) spores.

Based on the policy, observed features to be treated as special regions until demonstrated otherwise include gullies (taxon 2-4) and bright streaks associated with gullies, subsurface cavities, the subsurface below 5 meters, and confirmed/partially confirmed Recurrent Slope Lineae (RSL).[22] Note that Dundas (2020) discusses potential aeolian origins for RSL, but the lack of aqueous involvement in Martian RSL is not yet demonstrated.[23] Features that indicate a special region include groundwater, sources of methane, geothermal activity, and modern outflow channels. Observed features that require a case-by-case evaluation before being classified as a special region include dark streaks, "pasted-on" terrain, and candidate RSL.

Spacecraft-induced special regions were studied by Shotwell et al (2019) and considered by Meyer et al (2019).[24,25] Generally, a spacecraft-induced special region [e.g., from contaminated hardware in contact with a buried radioisotope thermoelectric generator (RTG)] is not problematic, unless proximity to a conduit to a natural "special region" (e.g., a subsurface liquid water environment) is possible. Missions are expected to perform analysis to demonstrate acceptably low risks for this "conduit" scenario.

[17] **https://www.physics.rutgers.edu/~ajbaker/honors292/COSPAR_Planetary_Protection_Policy_v3-24-11.pdf**

[18] Preventing the Forward Contamination of Mars. 2006. National Academies Press. Washington, D.C.: National Academies Press. Pg. 5. **https://nap.nationalacademies.org/catalog/11381/preventing-the-forward-contamination-of-mars.**

[19] Beaty, D.W., K.L. Buxbaum, M.A. Meyer, N. Barlow, W. Boynton, B. Clark, J. Deming, et al. 2006. "Findings of the Mars Special Regions Science Analysis Group." *Astrobiology* 6 (5): 56.

[20] Gerhard Kminek, John D Rummel, Charles S Cockell, Robert Atlas, N G Barlow, D W Beaty, W V Boynton, et al. 2010. "Report of the COSPAR Mars Special Regions Colloquium." *Advances in Space Research* 46 (6): 811–29.

[21] Rummel, John D., David W. Beaty, Melissa A. Jones, Corien Bakermans, Nadine G. Barlow, Penelope J. Boston, Vincent F. Chevrier, et al. 2014. "A New Analysis of Mars 'Special Regions': Findings of the Second MEPAG Special Regions Science Analysis Group (SR-SAG2)." *Astrobiology* 14 (11): 887–968.

[22] See footnote 21.

[23] Dundas, Colin M. 2020. "An Aeolian Grainflow Model for Martian Recurring Slope Lineae." Icarus 343 (June): 113681.

[24] Shotwell, Robert F., Lindsay E. Hays, David W. Beaty, Yulia Goreva, Thomas L. Kieft, Michael T. Mellon, George Moridis, Lee D. Peterson, and Nicolas Spycher. 2019. "Can an Off-Nominal Landing by an MMRTG-Powered Spacecraft Induce a Special Region on Mars When No Ice Is Present?" Astrobiology 19 (11): 1315–38.

[25] Meyer, Michael, Corien Bakermans, David Beaty, Douglas Bernard, Penelope Boston, Vincent Chevrier, Catharine Conley, et al. 2019. "Report of the Joint Workshop on Induced Special Regions." Life Sciences in Space Research 23 (November): 50–59.

COSPAR PP policy states, “Any region which is interpreted to have a high potential for the existence of extant Martian life forms is also defined as a special region.”[26] However, since we have no knowledge of how to determine the potential of locations to host unknown extant Martian life forms, we have no way to define them, except by reference to our knowledge of the terrestrial biosphere (and the water activity and temperature parameters that already apply).

The policy does not specifically address aspects of time/timeliness in the context of special regions. In the original 2006 SR-SAG discussions, the findings were framed in the context of “modern Mars,” with 500 years from now being the timeframe of consideration (based in part on this being the timeframe within which a meteorite would randomly land at a spacecraft location with a probability of 1×10^{-3}).

Further, the policy does not specifically address scale. Does the occurrence of a molecular scale liquid water film constitute a special region? Missions should address the significance of such phenomena in terms of the risk of harmful contamination.

Also, the policy specifically states that the water activity and temperature thresholds must be satisfied at the same time for a location to be considered a special region. However, it might be possible for terrestrial microorganisms to use “discontinuous” metabolism, sequestering water as it is available (e.g., from vapor) and using it metabolically at a later time (e.g., when the ambient temperature has risen).[27] Missions should address the significance of such phenomena in terms of the risk of harmful contamination.

2.1.5 Sample Return Missions

Planetary missions that return samples to Earth from solar system targets are assigned PP Category V. Sample return missions get both an outbound Category I-IV along with the inbound Category V for sample return. This category is further delineated into two subcategories consisting of Category V(u) for unrestricted Earth return and Category V(r) for restricted Earth return. The categories are assigned based on the risk of return samples containing anything that could cause adverse changes in the environment of the Earth. Several target bodies are already categorized as V(u) or V(r) in NPR 8715.24 Appendix C.[28] Missions returning samples from Earth’s Moon, Venus, or most asteroids and comets are Category V(u) and have no additional PP requirements for the returning sample. Missions returning samples from Mars, Europa, and Enceladus are categorized with V(r). A mission to another target body that is not already assigned a return category should present scientific evidence to demonstrate that the returned material will not result in any adverse changes in the environment of the Earth-Moon system to receive V(u) categorization. Category V(u) missions only require documentation with no further design, implementation, or operational PP requirements. In contrast, Category V(r) missions implement stringent controls to ensure that no uncontained returned material is released into the Earth-Moon system. *Chapter 8.0.0 Backward PP* provides more detail on the background and implementation of Category V(r) missions.

2.2.0 Horizontal and Vertical Mobility

Within the PP discipline, the phrase “horizontal and vertical mobility” is used to capture the concept that contamination spread at a planetary body can be in three dimensions, depending on the capabilities and operations of the spacecraft.

Given the intent of PP to avoid harmful contamination, the contamination threat for a target body through either horizontal or vertical mobility is always tempered by our knowledge (or lack thereof) of the landing/operating site, with the only modulator of contamination threat available being the cleanliness of the spacecraft hardware at the operating site. The primary targets of concern are considered individually below.

For Mars, horizontal mobility of a robotic spacecraft could come into play for potential interaction with special regions on the surface. Vertical mobility needs to be considered for access to environments where subsurface water or ices/brines may allow transport to depth over time. There are two depth levels to consider: below the reach of ambient

[26] “COSPAR POLICY on PLANETARY PROTECTION.” Page 6. **https://cosparhq.cnes.fr/assets/uploads/2024/07/PP-Policy_SRT_220-July-2024.pdf**.
[27] Rummel, John D., David W. Beaty, Melissa A. Jones, Corien Bakermans, Nadine G. Barlow, Penelope J. Boston, Vincent F. Chevrier, et al. 2014. “A New Analysis of Mars ‘Special Regions’: Findings of the Second MEPAG Special Regions Science Analysis Group (SR-SAG2).” Astrobiology 14 (11): 887–968. **https://doi.org/10.1089/ast.2014.1227**.
[28] **https://nodis3.gsfc.nasa.gov/displayDir.cfm?Internal_ID=N_PR_8715_0024_&page_name=AppendixC**

Galactic Cosmic Rays (GCR) at ~2 m depth, and at the level of subsurface aquifers that models show to be much deeper (order 100s of meters; really only a problem for deep drilling activities).[29] Previously, consideration had been given to contamination at the ~2.5 cm depth (e.g., from contamination buried as a result of regolith turnover by rover wheel tracking). Such buried contamination would not be a harmful contamination threat for any material buried in shallow subsurface locations unless occurring in a special region.

High levels of radiation at surface of Europa make the environment inhospitable to earth microorganisms, but radiation levels are not uniform.[30,31] It is possible through access into cracks and fissures, turnover of "young" surfaces, deliberate drilling/burrowing, or hardware impact below the level of incident radiation for viable contamination to be transported through vertical mobility. Missions should evaluate risk of harmful contamination based on conservative evaluation of the best available scientific data for missions below the Europan surface, especially if targeting access to subsurface aqueous liquid features such as lenses or a subsurface ocean.

The radiation environment at Saturn's moon Enceladus is less than that found on Europa, so the radiation there may not be sufficient to sterilize spacecraft hardware in the typical timeframe of a planetary mission (10s of years).[32] For this reason, and the emission and turnover of material at the south polar region, it might be expected that missions employing either horizontal or vertical mobility would require stringent cleanliness to avoid introduction of harmful contamination into a habitable location.

Transport Mechanisms

The spread of terrestrial contamination from a source location at or on a planetary body can occur through multiple pathways. These transport mechanisms need to be accounted for and evaluated in the development of a PP approach in both the deployment and operation of a spacecraft mission.

When considering harmful contamination, at least in principle, this can occur through the release of a single viable organism into an environment. Whether or not harmful contamination does occur is dependent on the presence/availability of a transport mechanism from the release location and the existence of a habitable environment or other location at which harmful contamination can occur, such as lunar PSRs.

Spacecraft Release

The initial release of contamination from a spacecraft is usually the first introduction into the environment of a solar system body from which further transport can occur. Examples include nominal and non-nominal deployments, nominal and non-nominal fluid release, nominal operations, and non-nominal events such as hardware breakup.

Impact/Sputtering/Scouring/Gardening-Type Processes

Contaminants on and in spacecraft hardware can be mobilized by impact processes at a variety of different scales. This includes, for example, spacecraft engine plume impingement during landing/launching events, removal related to weather events (e.g., Mars dust storms), and scouring by ice crystals during an Enceladus plume fly-through.

Aeolian Processes

In planetary environments with an atmosphere (principally Mars, from a PP perspective), contamination dispersion by the wind is generally only a consideration for short distance (from contaminated hardware to a sensitive location) or high bioburden (crewed surface spacecraft operations) situations where dispersion of viable organisms to remote locations is a significant threat.

[29]Dartnell, L. R., L. Desorgher, J. M. Ward, and A. J. Coates. 2007. "Modelling the Surface and Subsurface Martian Radiation Environment: Implications for Astrobiology." Geophysical Research Letters 34 (2). **https://doi.org/10.1029/2006gl027494**.

[30]Wesley Patterson, G., Chris Paranicas, and Louise M. Prockter. 2012. "Characterizing Electron Bombardment of Europa's Surface by Location and Depth." Icarus 220 (1): 286–90. **https://doi.org/10.1016/j.icarus.2012.04.024**.

[31]McCoy K, DiNicola M, Everline C, Burgoyne H, Reinholtz K, Clement B, *Europa Clipper planetary protection probabilistic risk assessment summary*, Planetary and Space Science, 2021

[32]Atwell, William, Brandon Reddell, and Paul Boeder. 2007. "A Comparison of the Radiation Environments in Deep Space." SAE Transactions 116: 133–39. **https://www.jstor.org/stable/44719451**.

Liquid Flow Processes

Liquid flow processes, particularly aqueous, have the potential to transport a single organism from an uninhabitable to a habitable environment. This can occur at multiple scales, so probability of occurrence together with outcomes in the timescale of relevance to PP needs to be considered.

Gravitational Processes

At Enceladus, contamination from orbiting spacecraft has the potential to be deposited at geologically active locations (such as the south polar “tiger stripes”) and has the potential to then be transferred to interior locations that might be habitable. Other similar scenarios can be envisaged (e.g., at Europa).

2.3.0 Example Categorization Scenarios

The following fictitious examples are provided to illustrate the process of PP mission categorization. OPP maintains a public list of all NASA missions and their categorizations on the OSMA Web site, which can provide helpful references and examples from actual NASA missions.

Example Mission 1

A mission will be exploring Earth’s Moon by landing a spacecraft lander on the surface outside a PSR, deploying a rover, and having a rover explore mostly outside of the PSR with commands sent from the lander. If the rover is in good health, the mission plans to have the rover spend a brief amount of time (less than 10% of the total mission time) exploring inside the PSR before returning to the lander. The final end-of-mission location for the rover will be at the lander site.

Categorization: Two elements are performing different operations at Earth’s Moon. Earth’s Moon is a Category II target body but has two subcategories depending on the exploration to be conducted. The lander is outside a PSR and is assigned a PP Category IIa. While the majority of the rover exploration will be outside the PSR, the fact that the mission plans to have the rover explore inside the PSR drives the categorization of the rover to a PP Category IIb.

Example Mission 2

A mission plans to send an orbiter to Titan to map hydrocarbons and liquid water on the surface. The orbiter will perform flybys of Venus and Earth before arriving at Titan. The mission plans for the orbiter to remain in orbit for three years. If the orbiter is healthy, the mission may be extended for additional operational years. The end-of-mission disposal for the orbiter is uncontrolled burnup and breakup in Titan’s atmosphere.

The scientific community is very excited for this proposed mission, but some are concerned about the uncontrolled entry into Titan’s atmosphere. The mission science and engineering team holds a series of reviews with the scientific community to further explain the details of the mission and the contamination risk analysis, which shows they can meet the remote potential for contamination definition of NASA-STD-8719.27. An independent science team also reviews the analysis and concludes the risk of contamination for Titan is low.

Categorization: Titan is a Category II* target body. This sets into motion the decision tree analysis of Figure 6. The mission spacecraft is an orbiter; however, the mission team demonstrates to the scientific community that the potential for contamination of Titan is remote. Therefore, the mission is assigned a PP Category II mission. If the potential for contamination is analyzed or determined, the mission is assigned a PP Category III mission. The mission must also factor in the flybys occurring in the trajectory for the spacecraft to get to Titan. The flyby of Earth is not an issue, and Venus, being a Category II target body, has the same sensitivity as the current categorization of the mission. Thus, the flybys have no impact on the categorization, and the mission would remain as a PP Category II mission.

Example Mission 3

A mission proposes to send an orbiter to a small body and needs to perform a Mars gravity assist as part of the mission design. The small body is a rocky, S-type asteroid predominately consisting of iron silicates. After the successful Mars gravity

assist, the orbiter will then take two years before it can perform a stable orbit injection to study the small body. The mission will conduct visual and scientific observations of the body to determine its composition and map safe landing sites for follow-on landed missions. The orbiter will remain in orbit for two years during the nominal mission, where an extended mission may be requested, or the orbiter may be safely disposed of in a disposal orbit.

Categorization: A rocky, S-type asteroid is a Category I target body. However, the mission needs to perform a Mars gravity assist, and Mars is a Category III target body for a flyby. For this mission, given that Mars is the most stringent body encountered, it is assigned a PP Category III mission categorization.

Example Mission 4

A mission will be sending two elements on the same spacecraft and launch vehicle to explore Mars. Once the spacecraft reaches Mars, an orbiter and a lander will be deployed. The mission plans for the orbiter to remain in orbit for five years, providing communications to the lander and obtaining additional science data from Mars. The team plans to request extended missions as long as the orbiter remains healthy. The lander will land on Mars outside of any special regions and will deploy instruments on an instrument deck to record Mars' seasonal weather data. The mission team hopes the lander will obtain at least three years of weather data before the solar panels become covered with too much dust to adequately charge the on-board batteries. For the end-of-mission plans, the lander will remain in place at the landing site while the orbiter will sundergo an uncontrolled burnup and breakup in the Mars atmosphere.

Categorization: There are two elements performing different operations at Mars, so there will be two PP mission categorizations. The orbiting element is assigned a PP Category III mission categorization. The lander will not be accessing a special region nor will it be returning samples from Mars. As a mission collecting weather data, it will not be performing life detection investigations. The lander is assigned a PP Category IVa mission categorization.

Note: *The orbiter and lander will be on the same spacecraft but may have different cleanliness requirements stemming from the different categorizations. The mission team must ensure that the cleanliness of the more sensitive lander hardware is maintained through proper PP implementation practices.*

Example Mission 5

A mission proposes to launch a spacecraft to collect samples of a comet's tail and return those samples to Earth. The proposed trajectory is a heliocentric orbit that would bring the spacecraft around the Sun and past Earth en route to the comet. After sample collection and return to Earth's orbit, the spacecraft sample canister is deployed by the spacecraft, and the orbiting spacecraft will burn up in Earth's atmosphere upon re-entry. The sample canister will land at the Utah Test and Training Range, near the U.S. Army Dugway Proving Ground, for retrieval by the mission team.

Categorization: The proposed mission will have two mission categorizations. The forward portion of the mission plans for a heliocentric trajectory where only the Earth and Sun are encountered on the way to the comet, and the comet is a Category II target body. The forward portion of the mission is assigned a PP Category II mission categorization. Since the mission is returning samples of the comet to Earth, the mission would also be assigned a PP Category V(u) unrestricted Earth return for the backward PP mission categorization. No additional PP requirements would apply for the backward portion of the mission other than documentation of the categorization.

Example Mission 6

A mission proposes to send a lander to Mars, where a helicopter is deployed to take samples of the Martian regolith no deeper than 6 cm and will not be accessing special regions. The helicopter will return 10 tubes the size of ballpoint pens to the lander, where a mechanical arm will load them into a rocket for return to Earth. The helicopter will continue to explore the surface of Mars until the battery runs out.

Categorization: The proposed mission will have two mission categorizations. The forward portion of the mission where a lander deploys a helicopter to collect samples of the Mars regolith is assigned a PP Category IVb mission categorization. The helicopter and lander are not performing life detection investigations on Mars and not accessing special regions, but the helicopter is collecting samples for return to Earth, which invokes the IVb subcategory. Since the mission is returning samples

of Mars to Earth, the mission is also assigned a PP Category V(r) restricted Earth return for the backward PP mission categorization. This mission would follow Section 5 of NASA-STD-8719.27 to preclude backward contamination of Earth upon sample return.

Example Mission 7

A mission is proposing two orbiters: one that will go to Earth's Moon and the other to Mars. Both missions have a desire to get detailed imaging of each target body and so will have lower orbits. For Earth's Moon, the nominal mission is planned for two years of imaging of the polar regions, then final mission disposition into the Earth's Moon. In the mission to Mars, close flybys (~150-200km) are needed to collect the science data. After the planned two-year mission, the orbiter will then serve as a communication relay for at least another five years. The mission intends to stack up the spacecraft where the Earth's Moon orbiter is released within the first days of launch and the remaining Mars orbiter and upper stage of the rocket maintains the Mars trajectory.

Categorization: The proposed mission will have two mission categories. For the orbiter going to the Earth's Moon, it is assigned a PP Category II. With the low orbits and short nominal mission plans, further evaluation is necessary to determine if additional requirements apply to the mission similar to a PP Category IIa or IIb lander mission. The Mars orbiter is assigned a PP Category III with bioburden constraints due to likely not being able to meet orbital lifetime probability requirements because of the low (~<450km) passes. Due to the Mars orbiter needing to adhere to bioburden cleanliness, further contamination analysis and bioburden cleanliness requirements may be imposed on the Earth's Moon orbiter.

2.4.0 In-Mission Operations for PP on Interplanetary Missions

PP intends to protect science at the target bodies for the period of biological exploration, which likely extends beyond any given mission lifetime. NPR 8715.24 contains a provision to ensure PP concerns continue to be addressed during the operations of the mission (the period between completion of the post-launch report and the end-of-mission report).

Section 3.3.3 of the NPR states, *"During mission operations, the NASA Project Manager, in coordination with the Project-Level SMA TA, shall notify the OPP of any discoveries or anomaly events relevant to planetary protection."*

Given this requirement, project management should make available the resources needed to address PP constraints in the following two topic areas:

1. Anomaly Events

It is relatively common for unexpected events to occur during the mission, and some of these can affect the ability of the mission to comply with existing PP categorization. For example, in the early phases of a Mars mission, anomalies that affect navigational accuracy or the ability to perform trajectory correction maneuvers (TCMs) may affect compliance with a probability of impact requirement. Further into a mission, anomaly events in orbit at the target, or after deployment to the surface, can similarly affect other compliance requirements. To avoid a harmful contamination event and maintain compliance, missions should be monitoring components, systems, and parameters. Single events that could jeopardize compliance include failures in systems for propulsion, guidance and navigational control, avionics, and telecom.

Monitoring of factors affecting PP compliance should be part of project and mission management, with processes such as the Performance/Failure anomaly Report (PFR) and Incident Surprise Anomaly (ISA) providing input. Anomalies such as uncommanded guidance, navigation or control system side swaps of redundant/multi-fault tolerant systems, uncommanded entry into "safe modes," and anything that affects a probability of impact estimation should be assessed for their impact on PP compliance. Events such as a single reaction wheel failure in a 5-wheel system, while reportable as it reduces the redundancy of the system, might be considered less of a threat to compliance due to that redundancy.

2. Discoveries

PP constraints are based on "current scientific consensus regarding the destination and mission operations, informed by COSPAR policy and guidelines, advice from the NASEM, and other expert advice."[33] In rare cases (although usually regarding

[33]NPR8715.24, Section 1.1.2. **https://nodis3.gsfc.nasa.gov/displayDir.cfm?t=NPR&c=8715&s=24**

the most sensitive life detection concerns), the discovery of new information during a mission can change the categorization of targets either for itself or for future missions. For example, the confirmation of a shallow subsurface ocean at Europa,[34, 35] led to the Galileo Mission being actively terminated at Jupiter rather than being allowed to be decommissioned elsewhere in the Jovian system. Similarly, the Cassini Mission was terminated at Saturn rather than being allowed to be decommissioned in Saturnian orbit in order to protect Enceladus from contamination after discovery of plumes and the tiger-stripe surface features there that could indicate a habitable environment.[36]

For Mars surface missions, discussions were held on potential responses if a mission encountered a special region (a location habitable to terrestrial organisms). PP's response to such incidents is based on a case-by-case review. However, for such incidents to be reported, the surface science team would need to include a trained representative to identify and communicate any changes from the baseline/approved plan.

3.0.0 Forward Planetary Protection

Navigation Summary: *Chapter 3 discusses approaches for determining the risk of forward contamination at a target body. This chapter is most applicable to Category II, III, and IV missions.*

Enabling current and future science by preventing harmful contamination during spacecraft activities is the objective of forward PP. Forward PP guidelines are established through scientific consensus and are determined by the risk of impacting life detection or experiments seeking to understanding origins of life. Projects and missions may employ a combination of implementation techniques to comply with forward PP guidelines, including bioburden management, inadvertent impact, trajectory biassing, cleanroom assembly, and organic inventory and archiving.

3.1.0 Guidelines for Forward Contamination Mitigation

Introduction

The Forward Contamination Risk Assessment Framework provides a means for assessing the effectiveness of proposed procedures for limiting forward contamination. As a follow-on to this framework, guidelines were developed to guide mission designers and providers in thinking strategically and considering the important factors for limiting forward contamination. These considerations should be made when developing, establishing, and implementing PP procedures for missions exploring solar system bodies. The four guidelines are:

1. Understand the contamination potential of the target solar system body to be explored (Figure 7).
2. Understand the cleanliness of the hardware upon arrival at the target solar system body (Figure 8).
3. Understand and mitigate the potential for the hardware to contaminate the target solar system body (Figure 9).
4. Understand and mitigate the potential for the hardware to contaminate other solar system bodies (Figure 10).

In the context of these guidelines, "mission" refers to NASA and NASA-partnered missions, conducted at a solar system body. "Mission hardware" refers to the spacecraft or other equipment used in missions. These guidelines are designed to be adaptable to and reflective of future developments in space exploration of new destinations, biological and other scientific discoveries, technologies and approaches to reduce the biological contamination of solar system bodies, capabilities of private sector participants, and international activities.

[34]Preventing the Forward Contamination of Europa. 2000. Washington, D.C.: National Academies Press. **https://doi.org/10.17226/9895**.
[35]Carr, M. H., M. J. Belton, C. R. Chapman, M. E. Davies, P. Geissler, R. Greenberg, A. S. McEwen, et al. 1998. "Evidence for a Subsurface Ocean on Europa." Nature 391 (6665): 363–65. **https://doi.org/10.1038/34857**.
[36]Hansen, C. J. 2006. "Enceladus' Water Vapor Plume." Science 311 (5766): 1422–25. **https://doi.org/10.1126/science.1121254**.

Understand the Contamination Potential of the Target Solar System Body To Be Explored

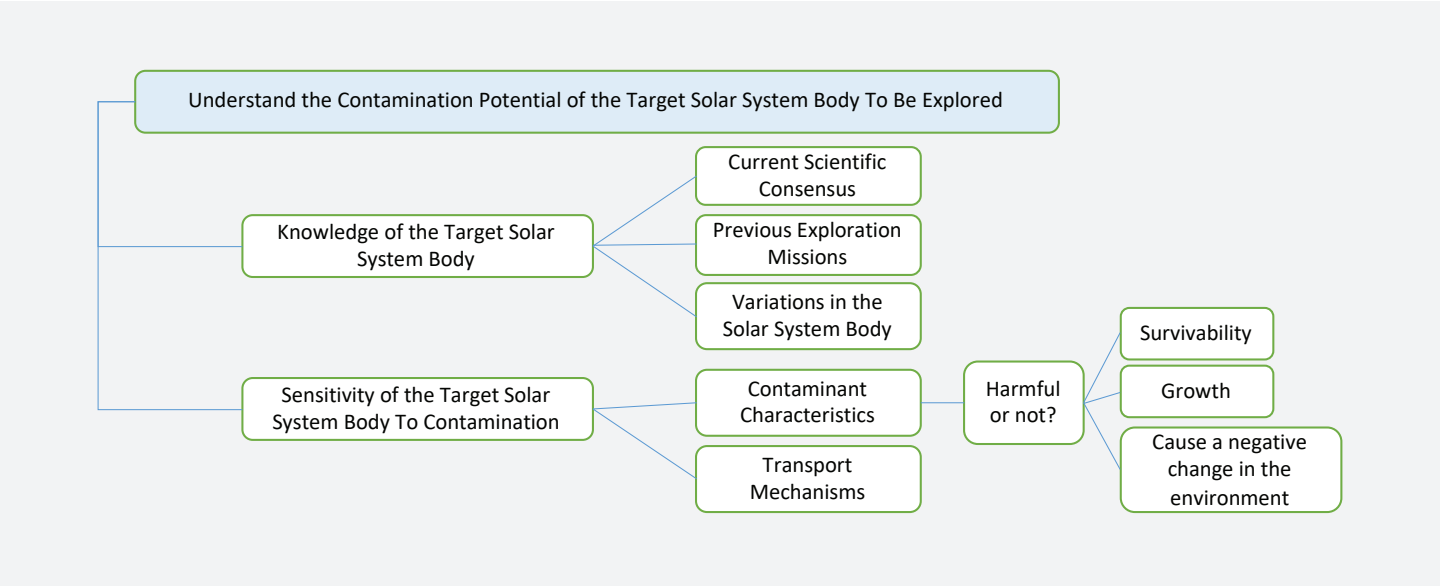

Figure 7: *Guidelines To Understand the Contamination Potential of the Target Solar System Body To Be Explored*

To prevent harmful biological contamination of solar system bodies during the exploration activities of a mission, the sensitivity of the target solar system body (destination) to terrestrial biological contamination and the potential for that body to be contaminated should be understood. This establishes the baseline knowledge of the pristine solar system body and enables contamination risk assessments of proposed exploration activities to be performed, which are discussed in *Section 3.2.0 Forward Contamination Risk Assessment Framework*.

Knowledge of the Target Solar System Body

Considerations for gaining knowledge of the target solar system body to be explored include: the current scientific consensus regarding the solar system body, the locations and types of investigations of the solar system body that were previously explored, and the temporal variation of the solar system body.

Existing knowledge and significant new scientific discoveries made and accepted as current scientific consensus should be considered to appropriately design missions for successful exploration. Current scientific consensus refers to the collective judgment, position, and opinion of the international space science community and may impact considerations for the entire mission lifecycle, from design and operation through mission disposal. Sources of information on current scientific consensus for solar system bodies include COSPAR, NASEM (or National Academies) and their Committee on Planetary Protection (CoPP), and peer-reviewed scientific journals. Examples of pertinent NASEM reports on PP are provided in *Appendix 2: National Academies Reports*. Technical working groups for scientific research communities such as the Lunar Exploration Analysis Group (LEAG), MEPAG, Outer Planets Assessment Group (OPAG), or organizations developing technical standards such as the International Organization for Standardization (ISO) and American Society for Testing and Materials (ASTM) are resources for participating in the scientific consensus process. Examples of technical standards that may be helpful for implementing PP practices are provided in *Appendix 4: Modeling the Probability of Contamination for NASA Missions*.

Knowledge of the target solar system body may also be obtained from previous observation studies and exploration missions. Initial knowledge of a solar system body is typically gained from scientific observations from Earth or observation platforms launched from Earth. Exploration missions to the solar system body build upon this knowledge by orbiting the body and making observations with orbital assets, exploring specific areas on the surface, or exploring volumes of the body by exploring in three dimensions, such as accessing the subsurface. Scientific consensus also takes into account varying scales relevant to biological processes to include a range of global phenomena down to microscales of microbial transport and interactions. Mission developers should be aware of and consider information learned from previous observation studies and exploration missions as well as current missions to the target solar system body.

Variations in the target solar system body, such as temporal variations (e.g., seasonal variations or orbital variations) should also be considered. Such variations can impact the environment to be explored by causing extreme temperatures above or below the surface, activity at or within the target body (e.g., dust storms, active liquid plumes, or volcanic activity), or other changes that could affect exploration missions during the mission lifetime. These variations can negatively impact mission hardware reliability and potentially increase the ability to transport contaminants from the hardware to the solar system body environment.

Understand the Sensitivity of the Target Solar System Body To Contamination

The sensitivity of solar system bodies to potential biological contamination can vary greatly between solar system bodies. For example, Mars has different sensitivity to contamination than Earth's Moon. Saturn's icy moon Enceladus has active liquid plumes with unique considerations compared to the rocky and dusty surface of Mars. For this reason, COSPAR categorizes solar system bodies into five categories based on the sensitivity of the solar system body to biological contamination.[37] NASA has also adopted these five PP categories into NASA policy for solar system exploration as defined in NPR 8715.24[38] and described in Section 2 of this handbook. The potential for a biological contaminant to cause harmful contamination at a target solar system body depends on the characteristics of the contaminant to survive, grow, and potentially cause a change in the environment of the solar system body. The types of life that can survive extreme environments on Earth as well as survive in the space environment should be considered when determining the probability of biological contaminant survival and growth in the environment of the target solar system body.

The potential for a biological contaminant to cause harmful contamination to the environment of the target solar system body also depends on the potential for contaminants to be transported from hardware surfaces to sensitive environments. Considerations should be made to understand potential transport mechanisms for contamination that may exist when exploring a solar system body (see *Section 2.2.0 Horizontal and Vertical Mobility*). These mechanisms include the geophysical, global, and macro-scale phenomena that can potentially transport and distribute contaminants through interactions with solids, liquids, and gases on the target body. For example, dust storms on Mars may cause particles of regolith to scour hardware surfaces, remove biological contaminants, and carry them to other locations. Drilling into the subsurface may introduce contaminants into underground aquifers through interactions with drilling equipment surfaces and aquifer fluids. These considerations will require knowledge of the target solar system body as discussed above.

[37] **https://cosparhq.cnes.fr/scientific-structure/panels/panel-on-planetary-protection-ppp/**
[38] **https://nodis3.gsfc.nasa.gov/displayDir.cfm?t=NPR&c=8715&s=24**

Understand the Cleanliness of the Hardware Upon Arrival at the Target Solar System Body

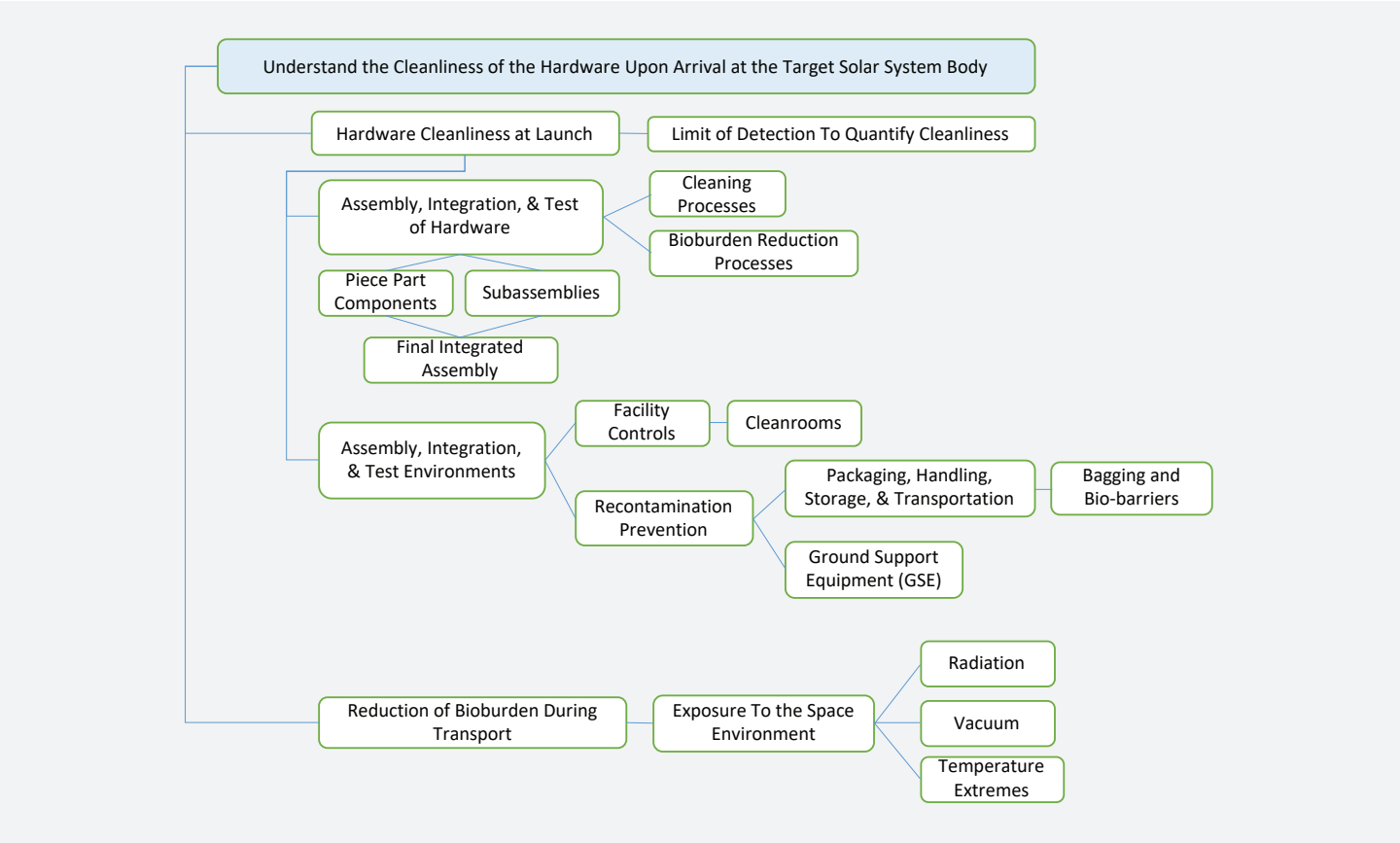

Figure 8: *Considerations To Understand the Cleanliness of the Hardware Upon Arrival at the Target Solar System Body*

Once the contamination potential of the target solar system body is understood, the next step is to understand the cleanliness of the mission hardware upon arrival at the destination of the target body. This depends on the cleanliness of the hardware at launch and any reduction of bioburden on the mission hardware during space flight transport to the destination.

Understand the Cleanliness of the Hardware at Launch

Several different methods may be used to determine the cleanliness of mission hardware during assembly, integration, and test, and the limit of detection of any methods used to quantify hardware cleanliness should be understood. This is an important consideration because a method that cannot adequately detect biological contamination may result in false confidence in the cleanliness of the hardware. Conversely, hardware surfaces may be extremely clean with low biomass, making the ability to detect and quantify contamination challenging for routine contamination detection methods. Methods to quantify hardware cleanliness should be appropriate for the type of hardware and able to adequately detect biological contamination for the type of mission and solar system body to be explored.

Understanding and quantifying hardware cleanliness should start by considering the entire hardware supply chain, beginning with sourcing piece part components from suppliers, assembling and testing subassemblies, and concluding with the final integrated assembly. This involves cleaning critical hardware surfaces and volumes for piece parts, subassemblies, and the final integrated assembly and performing bioburden reduction processes where needed. DHMR of hardware or processing hardware with vapor hydrogen peroxide (VHP) or ethylene oxide (EtO) are examples of hardware bioburden reduction processes. Biological contamination control should be considered as early as possible in mission planning, and suppliers in the supply chain should be engaged in supporting the control of biological contamination.

Assembly, integration, and test environments can be sources of biological contamination. Facilities should be controlled through environmental controls (e.g., filtered airflow, temperature, or humidity), personnel controls (e.g., reducing the number

of people in the environment or garment requirements), and material controls (e.g., reducing materials that shed or generate contamination) to reduce the potential for biological contamination of mission hardware. Cleanrooms and clean zones are examples of facility controls that may be utilized in assembly, integration, and test environments to control particulate, molecular, and biological contamination of sensitive hardware.

Once the hardware is cleaned, efforts should be made to maintain the cleanliness of the hardware and prevent recontamination. Covering the hardware in clean bagging or utilizing bio-barriers during packaging, handling, storage, and transportation can protect the hardware from unclean or uncontrolled environments or from processes that are known to generate debris and contaminants. As an alternative, extremely sensitive components can utilize flight spares for testing and replace the component with a clean flight unit during late-stage integration (e.g., last steps of spacecraft launch preparations prior to launch vehicle integration). Additionally, considerations should be made for cleaning and maintaining ground support equipment (GSE) that interacts with mission hardware during assembly, integration, and testing. In some cases, the GSE may need to be as clean as the mission hardware to prevent the GSE from being a source of biological contamination.

Understand the Potential Reduction of Bioburden During Transit

After the launch of the mission hardware, additional bioburden reduction may occur due to exposure of the hardware to the space environment, including radiation, vacuum, and temperature extremes. However, the amount of bioburden reduction may need to be determined through experiments and analytical models to understand the microbial tolerance to the environmental modalities for specific biological contaminants and hardware surface materials and exposure. The scientific research, technical working groups, and organizations developing technical standards previously mentioned are resources for exchanging information on bioburden reduction processes and effectiveness of methods.

Understand and Mitigate the Potential for the Hardware To Contaminate the Target Solar System Body

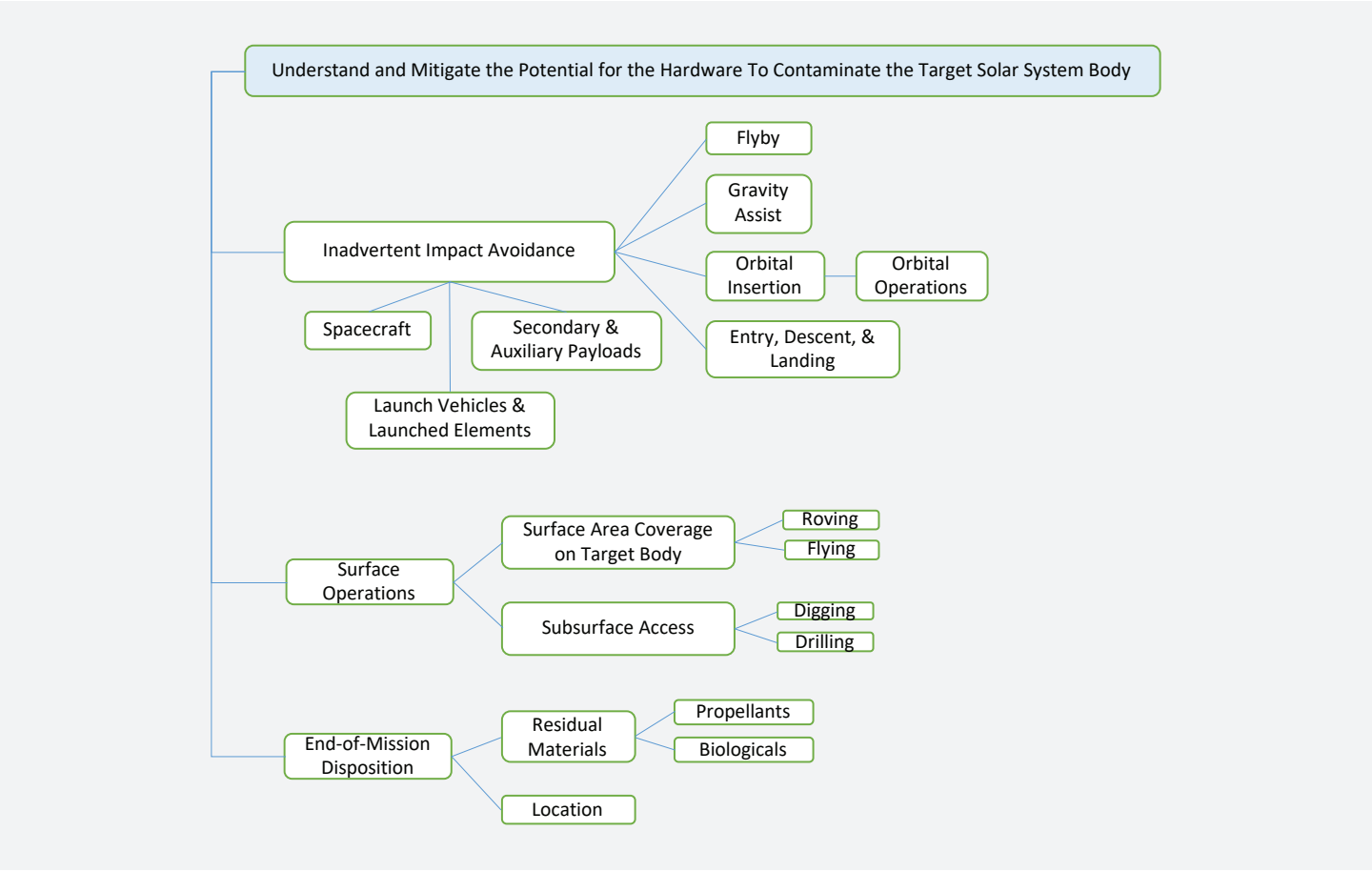

Figure 9: *Considerations To Understand and Mitigate the Potential for the Hardware To Contaminate the Target Solar System Body*

The potential for all launched elements to contaminate a target solar system body should be considered at all mission phases from launch, through operations at the target solar system body, to end-of-mission disposal of mission hardware. Depending on the potential for biological contamination in each phase, mitigation efforts may be needed.

Understand and Mitigate the Potential for Inadvertent Impact

Inadvertent impact of the target solar system body with launched hardware must be avoided, and COSPAR provides guidelines for probability of contamination of a solar system body by inadvertent impact.[39] These guidelines should be considered for all hardware with propulsion capability to reach the target solar system body and includes spacecraft, secondary and auxiliary payloads, launch vehicles, and launched elements. Additionally, all operations that may bring hardware within the gravity influence of the target body must also avoid inadvertent impact with the solar system body. Inadvertent impact should be considered for all missions conducting flyby; gravity assist; orbital insertion; orbital operations; and entry, descent, and landing (EDL) operations. Mitigation efforts such as contamination and collision avoidance maneuvers (CCAM) and trajectory biasing may be utilized to avoid inadvertent impact by launched elements. Inadvertent impact avoidance during hardware EDL at the target solar system body will depend on the reliability of the EDL system.

Understand and Mitigate the Potential for Contamination During Surface Operations

After the EDL phase is complete, surface operations on the target solar system body are the next phase to consider. This phase has a higher risk for biological contamination because the surfaces of the mission hardware interact directly with the environment. For mission hardware accessing the surface, the surface area covered by the hardware on the solar system body is a consideration, as more surface area accessed may increase the potential for contamination. A lander that remains in a single location may be a lower risk for biological contamination compared to mission assets with mobility, such as rovers and flyers (e.g., rotorcraft). The areas on the solar system body to be accessed by mobile assets should also be considered, especially if the mobile assets are to access highly sensitive areas that may have an increased probability for the habitability of terrestrial life. Additionally, considerations should be made for subsurface access by mission hardware on the solar system body through actions such as digging and drilling. Subsurface access may increase the potential for hardware surfaces to contact underground water or ice sources that are sensitive to biological contamination. Depending on the level of risk for each scenario, mitigation activities may be required to reduce the risk of biological contamination to the solar system body during surface operations. The process of developing mitigation activities may be guided by the performance measures of the risk-informed decision-making (RIDM) objectives' hierarchy of the forward contamination risk assessment framework for avoiding harmful biological contamination of explored solar system bodies. RIDM is discussed more extensively in *Section 3.2.0 Forward Contamination Risk Assessment Framework*.

Understand and Mitigate the Potential for Contamination During End-of-Mission Disposition

Finally, end-of-mission disposition options should also consider potential contamination vectors to the target solar system body over time. Residual materials that may remain at the solar system body may contribute embedded biological contamination as materials degrade and break down over time. For example, some propellants may contribute biological contamination if the propellant cannot be cleaned to remove biologicals before launch. Organic materials may also degrade and release volatiles into the environment, which can be a concern for conducting future science missions on certain solar system bodies. Both the COSPAR and NASA PP mission categories provide additional information on solar system bodies' sensitivity to these types of contaminants, and reporting of an organic inventory can help inform the science community of potential contaminants. Considerations should also be made for the final end-of-mission location of hardware in orbit or on the surface of a solar system body, and mitigation steps should be taken to avoid inadvertent impact or contact with potentially sensitive areas of the solar system body. Where applicable, analysis should be conducted for orbital assets to determine the breakup and burnup of mission hardware during de-orbit in the solar system body atmosphere. The final end-of-mission location of all hardware components should be reported to COSPAR for annual reporting to the Secretary General of the United Nations. The United Nations Office for Outer Space Affairs (UNOOSA) maintains an online index of objects launched into outer space.[40]

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[39]See Footnote 37.
[40]**https://www.unoosa.org/oosa/osoindex/index.jspx?lf_id=**

Understand and Mitigate the Potential for the Hardware To Contaminate Other Solar System Bodies

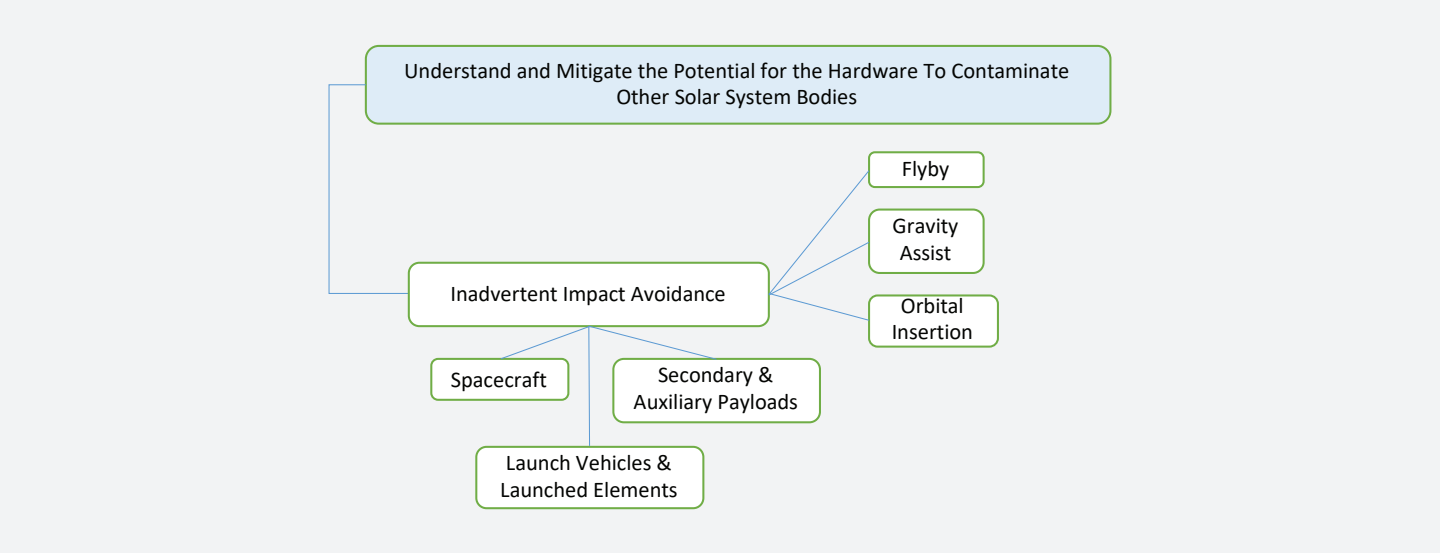

Figure 10: *Considerations To Understand and Mitigate the Potential for the Hardware To Contaminate Other Solar System Bodies*

Not only should mission hardware avoid inadvertent impact with the target solar system body, but also with all other solar system bodies. The same probability of contamination by inadvertent impact guidelines provided by COSPAR apply.[41] These guidelines should be considered for all hardware with propulsion capability to reach any other solar system body and includes spacecraft, secondary and auxiliary payloads, and launch vehicles and launched elements. If hardware has the capability of reaching other solar system bodies beyond the mission target body, this is also considered in mission classification. Operations that may bring launched hardware within the gravity influence of a solar system body through flyby, gravity assist, or orbital insertion must also avoid inadvertent impact with that solar system body. The same mitigation efforts of CCAM and trajectory biasing as described earlier may be utilized to avoid inadvertent impact by launched elements at other solar system bodies.

3.2.0 Forward Contamination Risk Assessment Framework

Introduction

A risk assessment framework for harmful forward biological contamination is described below to ensure NASA's PP policy is consistent with modern scientific and statistical practices and to assess the effectiveness of proposed mission implementation for limiting forward contamination. An RIDM approach was used to create the Forward Contamination Risk Assessment Framework (Figure 11) based on NASA's RIDM Handbook.[42] RIDM was chosen as a process because it acknowledges the key role that human judgment plays in decisions and incorporates a diverse set of performance objective and measures to help inform decision-making. Performance measure metrics are based on current scientific knowledge and each performance objective defines considerations to determine the probability of contamination. Together, these satisfy the objective of the risk assessment framework.

[41] See Footnote 37.
[42] **https://ntrs.nasa.gov/citations/20100021361**

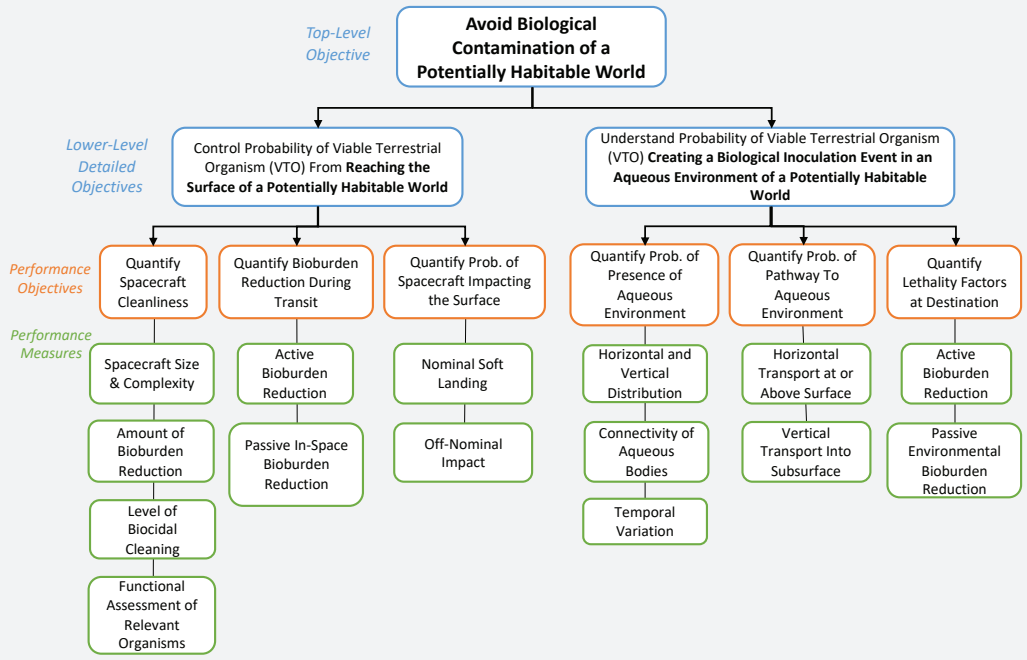

Figure 11: *Forward Contamination Risk Assessment Framework*

Figure 12 provides an example of an objectives and performance measures hierarchy that is created as part of the RIDM process. The process begins by defining the top-level objective, which states the goal to be accomplished. This objective may be further decomposed into distinct lower-level detailed objectives that provide more detail and clarify the top-level objective. Lower-level detailed objectives are further decomposed into a set of performance objectives. Each performance objective relates to a single facet of the detailed objectives and is quantifiable. Finally, performance measures are defined for each performance objective. Performance measures are the metrics that quantify the extent that a performance objective is fulfilled and provide the means to assess each performance objective.

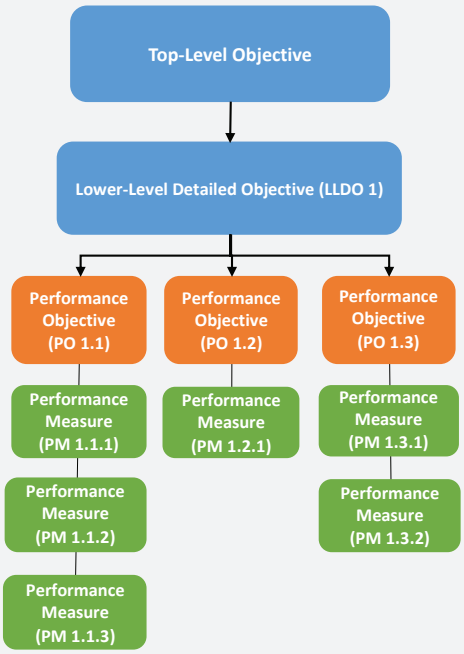

Figure 12: *RIDM Objectives and Performance Measure Hierarchy*

Forward Contamination Risk Assessment Framework

Top-Level and Detailed Objectives

The overall objective of the Forward Contamination Risk Assessment Framework is to avoid the biological contamination of a potentially habitable world to protect current and future scientific investigations when exploring solar system bodies. This objective is further defined by two lower-level detailed objectives (Figure 13). The first is to control the probability of a viable terrestrial organism (VTO) from reaching the surface of a potentially habitable world to an acceptable level. This probability is currently defined in the COSPAR policy for PP[43] and NASA's technical standard for PP, NASA-STD-8719.27.[44] The second lower-level detailed objective is to understand the probability of a VTO creating a biological inoculation event in an aqueous environment of a potentially habitable world.

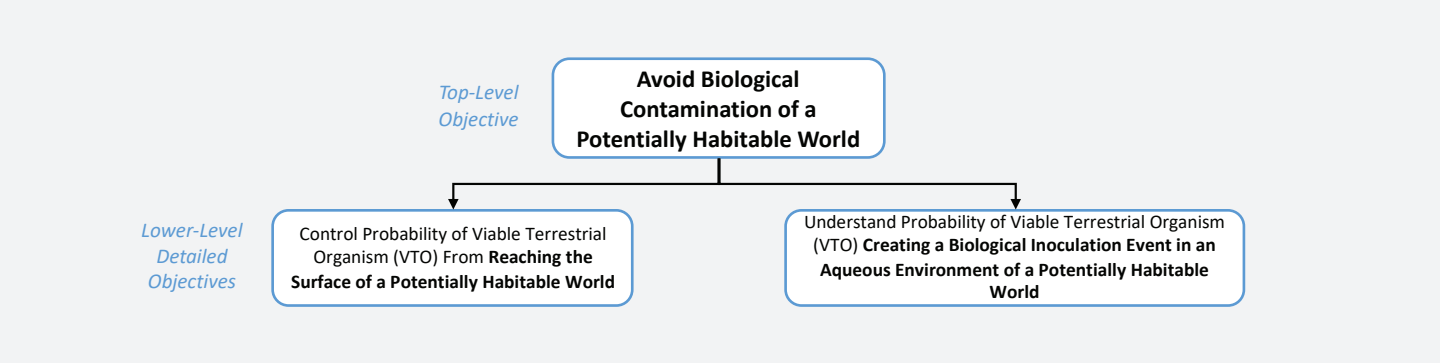

Figure 13: *Forward Contamination Risk Assessment Framework – Top-Level and Lower-Level Detailed Objectives*

Performance Objectives and Measures to Control the Probability of a VTO From Reaching the Surface of a Potentially Habitable World

The probability of a VTO reaching the surface of a potentially habitable world is controlled through three defined performance objectives: understanding and quantifying the cleanliness of the spacecraft prior to launch, quantifying the bioburden reduction that occurs during transit from Earth to the target solar system body, and understanding and quantifying the probability of the spacecraft impacting the surface of the solar system body (Figure 14). Performance measures are further defined to assess these performance objectives.

Spacecraft size and complexity are major factors in understanding and quantifying spacecraft cleanliness prior to launch. As a spacecraft increases in size and complexity of systems, the ability to keep critical surfaces clean and within biological cleanliness requirements for terrestrial organisms becomes more and more difficult. The amount of bioburden reduction through activities such as DHMR and biocidal cleaning of spacecraft surfaces during the assembly, integration, and test processes further quantifies spacecraft cleanliness. Additionally, genomic knowledge of relevant organisms can help to define the functional traits for terrestrial organisms, which may then be used to assess the potential viability in the habitable world to be explored. Further testing on Earth may be necessary to verify the genetic-based assessments of functional traits' inferred tolerance to extreme conditions on habitable worlds to be explored.

Once the biological cleanliness of the spacecraft is understood and quantified prior to launch, together with any anticipated launch recontamination, the bioburden reduction that may occur during spacecraft transit from Earth to the target solar system body can be defined. This bioburden reduction includes both active bioburden reduction that may take place onboard the spacecraft and passive in-space bioburden reduction. Active bioburden reduction techniques may include deliberate controlled exposure to radiation or chemical processing onboard the spacecraft. Passive bioburden reduction may involve relying upon exposing spacecraft surfaces to the temperature extremes, vacuum, and radiation environment of space to

[43] See Footnote 23.
[44] **https://standards.nasa.gov/safety-quality-reliability-maintainability**

further reduce bioburden.[45,46,47,48] However, both active and passive techniques would require verification during experiments on Earth or via in-space monitoring to quantify the amount of bioburden reduction that would take place during spacecraft transit.

The final performance objective is to understand and quantify the probability of the spacecraft impacting the surface of the potentially habitable world that is to be explored as the target solar system body. Spacecraft hardware surfaces will contact the surface of the target solar system body during nominal soft or hard landing processes, and this intentional contact may provide a transport path of a VTO to the surface of the target solar system body. However, a greater concern is off-nominal situations during transit, orbit, or landing that may create a hard impact on the surface. A hard impact may break open the spacecraft and expose internal surfaces that may contain greater bioburden levels or embedded bioburden to the target solar system body. The process of impacting may also drive spacecraft hardware surfaces into the surface of the target solar system body with greater force. Additionally, off-nominal impacts may be unpredictable and carry the risk of contaminating areas of higher sensitivity to biological contamination on the target solar system body. For both nominal soft landing and off-nominal impact scenarios, the amount of potential biological contamination reaching the surface should be analyzed to understand the probability of a VTO reaching the surface.

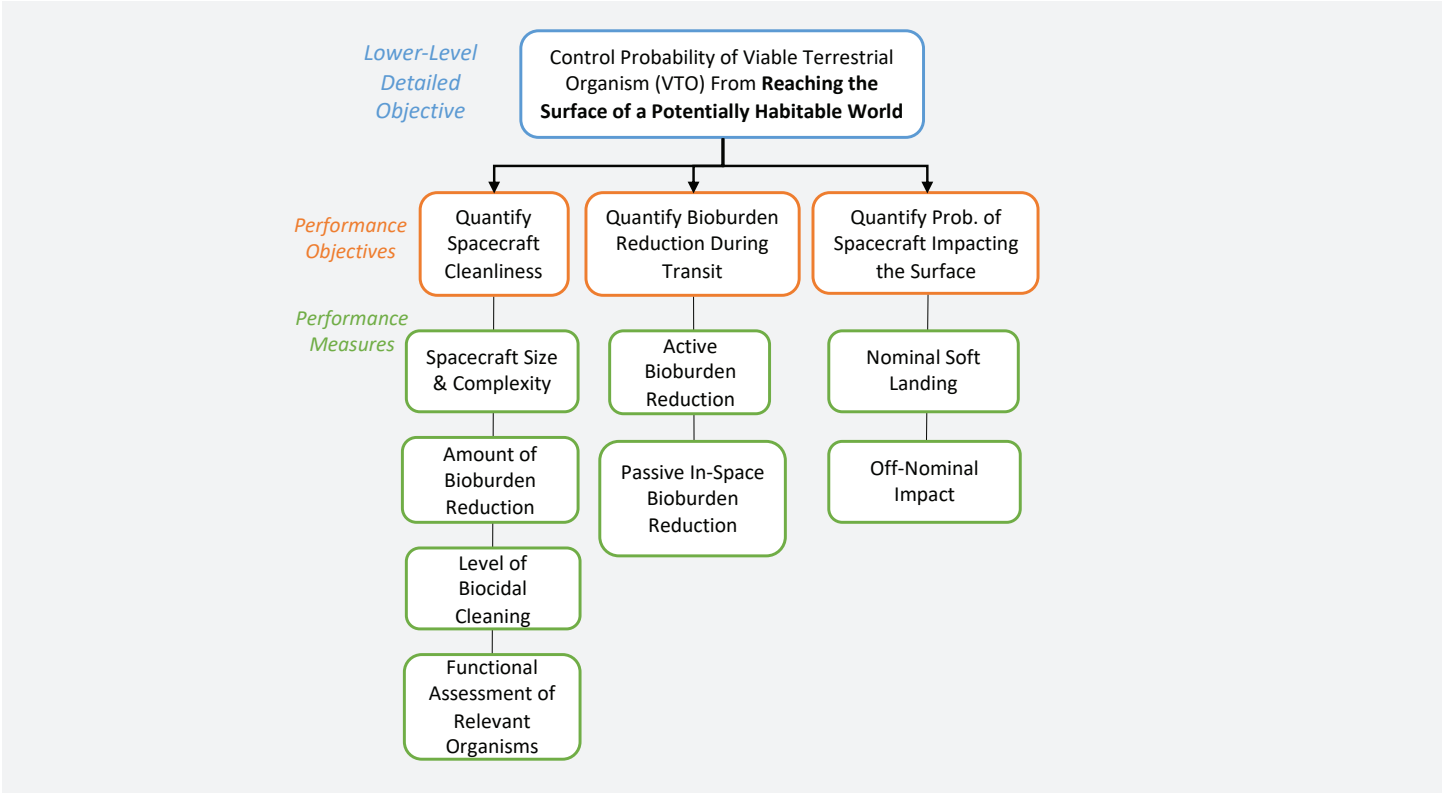

Figure 14: *Performance Objectives and Measures To Control the Probability of a VTO From Reaching the Surface of a Potentially Habitable World*

Performance Objectives and Measures to Understand the Probability of a VTO Creating a Biological Inoculation Event in an Aqueous Environment of a Potentially Habitable World

The second lower-level detailed objective is to understand the probability of a VTO creating a biological inoculation event in an aqueous environment of a potentially habitable world. This probability is further defined by the following performance

[45]Mileikowsky, C. 2000. "Natural Transfer of Viable Microbes in Space 1. From Mars to Earth and Earth to Mars." Icarus 145 (2): 391–427. **https://doi.org/10.1006/icar.1999.6317**.
[46]Pillinger, Judith M., C.T. Pillinger, S. Sancisi-Frey, and J.A. Spry. 2006. "The Microbiology of Spacecraft Hardware: Lessons Learned from the Planetary Protection Activities on the Beagle 2 Spacecraft." *Research in Microbiology* 157 (1): 19–24. **https://doi.org/10.1016/j.resmic.2005.08.006**.
[47]Matthiä, Daniel, Bent Ehresmann, Henning Lohf, Jan Köhler, Cary Zeitlin, Jan Appel, Tatsuhiko Sato, et al. 2016. "The Martian Surface Radiation Environment – a Comparison of Models and MSL/RAD Measurements." *Journal of Space Weather and Space Climate* 6: A13. **https://doi.org/10.1051/swsc/2016008**.
[48]Leshin, L A, A Yen, J Bomba, B Clark, C Epp, L Forney, T Gamber, et al. n.d. "SAMPLE COLLECTION for INVESTIGATION of MARS (SCIM): AN EARLY MARS SAMPLE RETURN MISSION through the MARS SCOUT PROGRAM." Accessed August 16, 2024. **https://www.lpi.usra.edu/meetings/lpsc2002/pdf/1721.pdf**.

objectives: understanding and quantifying the probability of an aqueous environment present on the potentially habitable world, quantifying the probability of the spacecraft hardware accessing pathways to the aqueous environment, and quantifying any biological lethality factors that may exist at the destination to be explored (Figure 15). Performance measures are further defined to assess these performance objectives.

The probability of an aqueous environment being present on the target solar system body to be explored depends on the current scientific knowledge about the solar system body. To understand and quantify this probability, data on the horizontal and vertical distribution of the aqueous environment(s) are required from scientific observations and measurements. The connectivity of aqueous bodies must also be understood to determine the risk of potential biological contamination and spread if spacecraft hardware contacts an aqueous body. Additionally, the temporal variation of aqueous environments on the target solar system body is important in understanding migrations of aqueous environments over time and changes in physical characteristics.

Once the aqueous environment on the target solar system body is understood, the probability of spacecraft hardware accessing pathways to the aqueous environment during exploration or mission operations can be quantified. Mission assets such as rovers and flyers (e.g., rotorcraft) may access these environments during horizontal transport at or above the surface. Mission assets may also explore vertically by drilling, digging, or tunneling into the subsurface of the solar system body. During both horizontal and vertical transport of mission assets, discussed in detail in *Section 2.2.0 Horizontal and Vertical Mobility*, the risk of biological contamination increases because surfaces of the mission hardware interact directly with the solar system body environment.

Finally, lethality factors for terrestrial biological contamination at the destination to be explored must be quantified. These lethality factors may occur due to active bioburden reduction processes or passive environmental bioburden reduction. As in the spacecraft transportation phase, active bioburden reduction processes on the surface may include exposure of critical hardware surfaces to radiation or chemical processing onboard the mission hardware. Passive environmental bioburden reduction may involve exposure of mission hardware surfaces to the natural environment of the solar system body to reduce terrestrial biological contamination. For example, high radiation environments may provide further bioburden reduction of mission hardware prior to surface or subsurface operations. However, as with in-space transport bioburden reduction (Figure 14), both active and passive techniques would require verification during experiments on Earth or in situ monitoring during mission operations to quantify the amount of bioburden reduction to be expected.

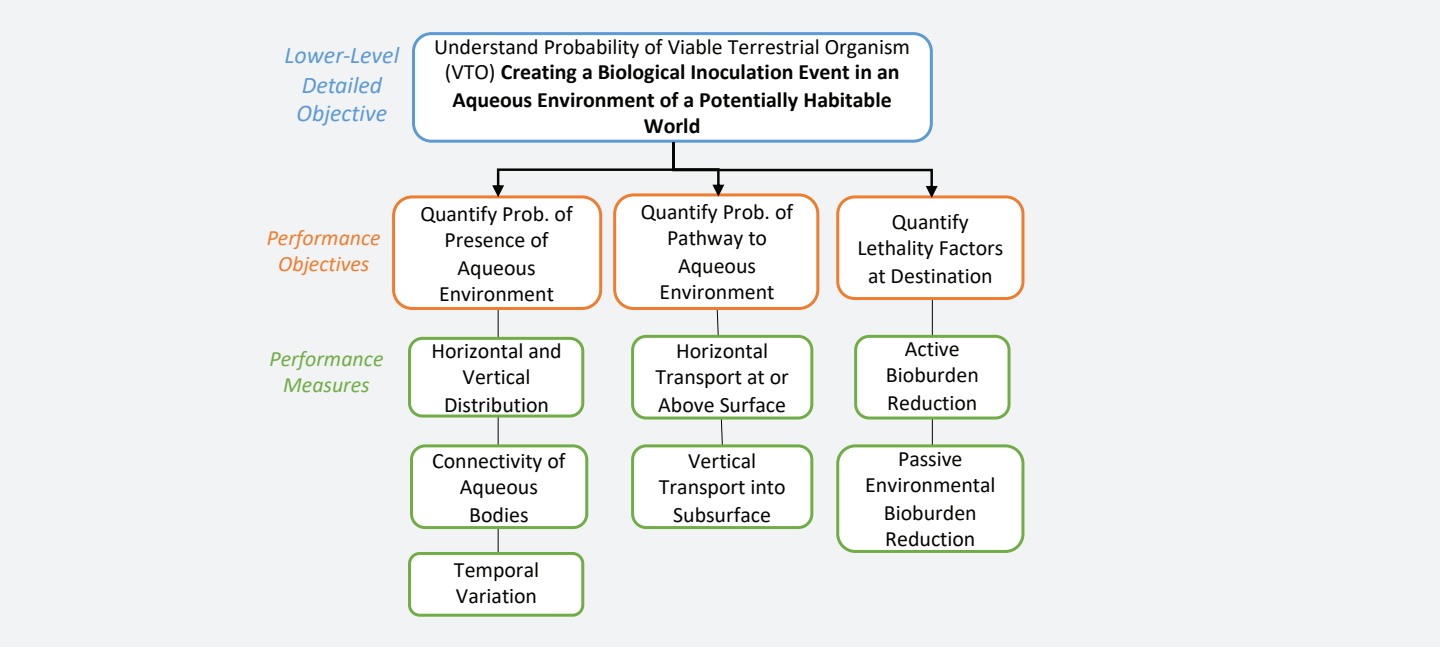

Figure 15: *Performance Objectives and Measures To Understand the Probability of a VTO Creating a Biological Inoculation Event in an Aqueous Environment of a Potentially Habitable World*

Mission PP Risk Analysis

The Forward Contamination Risk Assessment Framework provides the performance objectives and measures to help inform decision-making when designing missions that must avoid biological contamination of a potentially habitable world when exploring solar system bodies. Mission risk analysis consists of performance assessment supported by probabilistic modeling. The performance measures defined by the framework provide a means of quantifying performance so that alternatives in mission designs can be effectively compared. Missions must then perform a risk analysis of the alternatives that are identified as part of the assessment process. This analysis involves the probabilistic evaluation of each alternative's performance measures so that a final selection can be made. The Forward Contamination Risk Assessment Framework ensures a consistent approach is provided to all missions to assess and evaluate risk.

A risk assessment approach may be used throughout the project lifecycle when planning, developing, and applying a mathematical model to assess the probability of contamination. As a mission seeks to develop a risk assessment, there may be different modeling techniques, including Uncertainty Quantification (UQ) and traditional Probabilistic Risk Assessment (PRA) *(Appendix 3: PP Category II Mission Organic Inventory Template)*. Practical knowledge as to how these techniques are applied in the PP model development and how the models fit into the project lifecycle needs to be considered. Risk assessment can be applied to forward and backward contamination cases for all types of mission configurations (e.g., orbiters, flybys, landers, probes, etc.). *Appendix 4: Modeling the Probability of Contamination for NASA Missions* details some of the past mission models that were used for NASA missions, how requirements were interpreted and evaluated for use within models, and key behaviors of the probability of contamination uncovered by previous modeling ventures.

Development of an Assurance Case

Along with the risk assessment framework, an assurance case may be developed. Assurance cases are structured arguments based on logic and reason that provide the basis for the risk level associated with a particular event. The assurance case is meant to help decision-makers understand the PP model using all available information and form a position as to the risk of the mission being assessed. The details of what constitute an assurance case are still being developed on a case-by-case basis.

For this application, elements of an assurance case can be quantitative models to determine the probability of contamination and qualitative factors to interpret and contextualize the model. The latter may involve qualitative standards to be addressed by such things as Best Available Technology (BAT)[49] or an organization's design principles and practices. It may also include qualitative assessments of processes that are not well understood, such as time scales and transfer through the subsurface of other ocean worlds (e.g., Europa) to a body of liquid water. All modeling and the assurance case must be reviewed with stakeholders and decision-makers to determine if the risk of the probability of contamination assessment being wrong is adequately low. This review is intended to fill a critical part of the risk evaluation process since there can be significant uncertainty as to whether or not the probability of contamination is low enough to satisfy the PP requirement.

3.3.0 Inadvertent Impact at Mars for Lunar (Earth-Moon System) Missions

NASA-STD-8719.27 (Table 4.3) includes a requirement for Cat. II–V missions to demonstrate a 1.0×10^{-4} probability of inadvertent impact on Mars.

For many Earth-Moon system missions, potentially including missions to Lagrange point destinations and others, this can be achieved by demonstrating the physical limitations (e.g., spacecraft mass, delta V, fuel available, etc.) of the hardware mean the spacecraft is not capable of reaching Mars. If necessary, an analysis may be run using Monte Carlo or other analysis methods using variable inputs to derive probabilistic outcomes. A Monte Carlo analysis is expected for all spacecraft capable of crossing the orbit of Mars.

The background for this approach is that, without the propellant capability to reach Mars, any errant spacecraft is going to remain in heliocentric orbit in the inner solar system without the capability to get out to Mars' orbital distance, even in the

[49]The BAT does not address a specific level of risk assurance but is the conclusion of a selection process in which several technological alternatives are evaluated accounting for factors related to technology readiness levels, launched mass limits, operational environments, and other mission parameters.

event of the most catastrophic loss of control, coincidental with a trajectory toward Mars (which itself could potentially be an input for a probabilistic analysis if needed).

The propulsive capability threshold to reach Mars for PP purposes is given as a dV of 600m/s,[50] defined as the velocity change needed to sustain a transition from the top of Earth's gravity well (C3=0) to a Mars intercept orbit. All of the Artemis I cubesats (including the interplanetary Biosentinel and NEAScout missions) were below this capability threshold by at least one order of magnitude. For spacecraft close to/above this capability level (likely to be larger missions where doing the analysis is comparatively less of a burden), a Monte Carlo-type analysis should be the preferred tool. Mission design/architecture capabilities (e.g., use of Venus gravity assists or the ability to refuel the spacecraft in space) should also be taken into account when developing the Mars inadvertent impact approach.

3.4.0 Generalized 50-Year Probability of Impact Requirement Compliance for Mars Missions

Missions that fly by or are orbiting Mars, as well as associated mission hardware that leaves Earth's orbit (e.g., launch vehicle upper stage hardware) can meet the PP impact avoidance requirements of less than 1.0×10^{-2} for 20 years after launch and less than 5.0×10^{-2} for a period extending from 20 to 50 years after launch. *Appendix 5: Generalized Mars Mission 50-Year Probability of Impact Requirement Compliance* details an approach to calculating these probabilities using statistical formulation for calculating probability of impact. It also details the importance of considering both the nominal mission scenario and potential anomalous scenarios when assessing the impact probability.

If probability of impact for an orbiter or flyby mission cannot be satisfied with trajectory analysis, then a bioburden approach that uses a burnup and breakup analysis may be an alternative approach to explore to meet PP requirements (refer to the next section).

3.5.0 Burnup and Breakup

Burnup and breakup analysis can be leveraged to understand the forward terrestrial contamination potential of a target body. This analysis can be applied to orbiting spacecraft, upper stage launch vehicle hardware, or jettisoned spacecraft elements. This type of analysis is used to predict the amount of biological material delivered from an orbiting spacecraft to the target body for two purposes, as described in Section 4.5.4 of NASA-STD-8719.27:

1. Orbital decay to demonstrate compliance with bioburden requirements when spacecraft reliability is not enough to meet the 20- or 50-year probability of contamination of an orbiting Mars spacecraft
2. Predicting the biological contamination during a planned end-of-mission disposal, when the mission launches with more than 5 x 10^5 to standardized spores

This analysis is comprised of trajectory analysis, entry heating analysis, breakup analysis, thermal analysis, and ablation analysis.[51] The trajectory and breakup analysis should consider the latest atmospheric models and examine tumbling and stable aerodynamic entry cases for the spacecraft. Conservative bounding cases should be considered in the reporting of total bioburden where credible cases would result in the hardware remaining intact and/or receiving the "coldest" thermal analysis during entry heating. As the spacecraft enters the atmosphere and begins to heat up, key components are then tracked as additional nodes based on when the structural joints fail. Thermal and ablation analysis results are then used to compute the final bioburden by considering the time at temperature of each component to the time at temperature specification for both sterilization as well as surface and encapsulated heat microbial reduction.[52,53] Once a component reaches the required 500 °C for 0.5 sec for sterilization, typically the simulation of that node is completed. The resulting data from this analysis can then be applied to the mission's Planetary Protection Equipment List (PPEL) to predict biological

[50]This is a "minimum" number, somewhat below the notional 1040m/s given in e.g., **https://www.reddit.com/r/space/comments/29cxi6/i_made_a_deltav_subway_map_of_the_solar_system/?rdt=61535**, that is based on masses/solar distance. On that basis, 600m/s is a conservative case that will accommodate potential changes relative to orbital positions.
[51]Barengoltz, J., and J. Witte. 2008. "Planetary Protection Implementation on Mars Reconnaissance Orbiter Mission." Advances in Space Research 42 (6): 1108–19.
[52]NASA-STD-8719.27 §Appendix C 3.d.ii
[53]ECSS-Q-ST-70-57C Dry heat bioburden reduction for flight hardware (30 August 2013)

contamination. This can be used directly for reporting of compliance of missions if below the bioburden, or the data can be used strategically in the design phase of a mission so that a mission knows which components need to undergo further bioburden control prior to launch to meet its bioburden requirements. Figure 16 below shows an example of how burnup and breakup can be used to meet bioburden requirements in combination with standard bioburden estimate, bakeouts, and bioassays as needed to reach <5 x 10^5 spores.

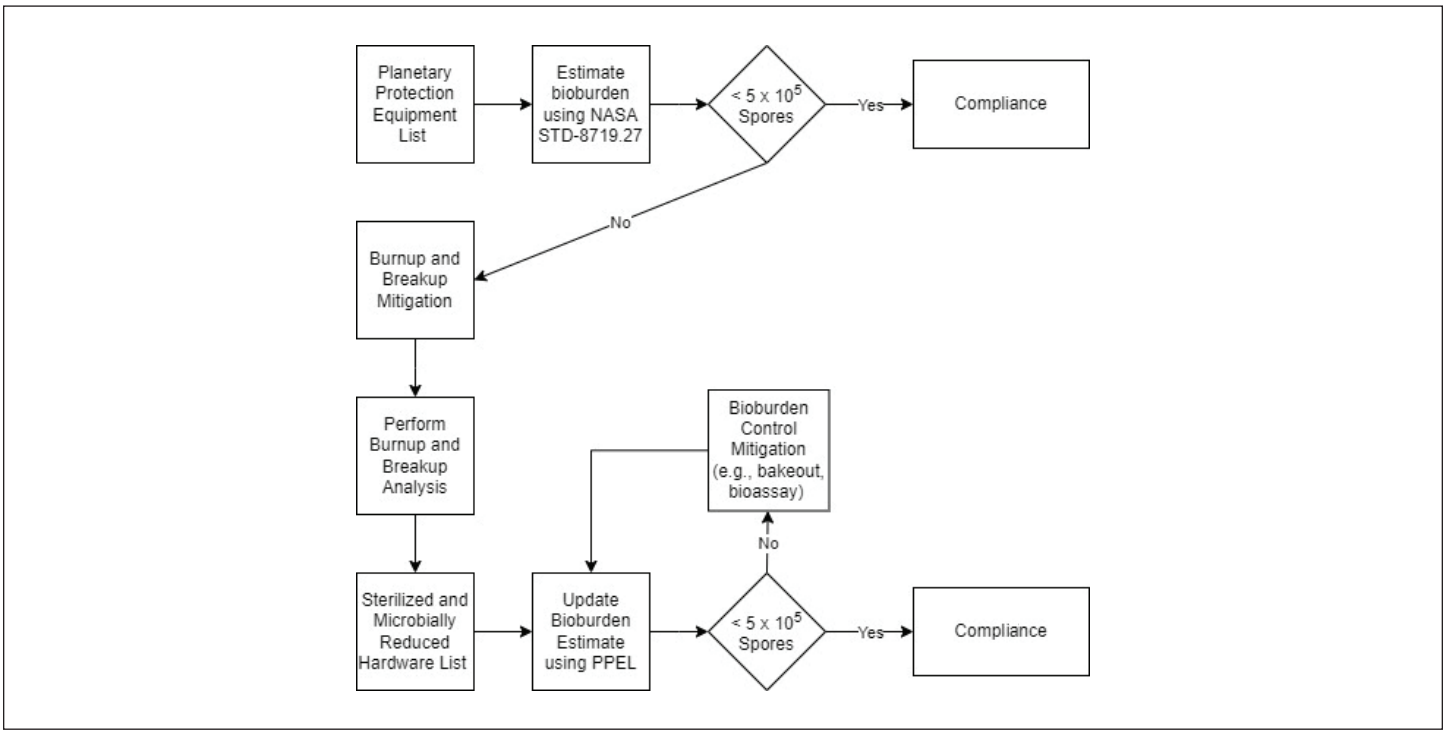

Figure 16: *An Example of How Burnup and Breakup Can Be Leveraged in an Orbiting Mission Utilizing a Bioburden Approach in the Design Phase of a Mission*

3.6.0 Launch Recontamination Analysis

For PP Category III and IV bioburden-controlled missions, a launch recontamination analysis may need to be conducted to assess the impact the launch will have on the spacecraft "at launch" bioburden. Simply put – what contribution, if any, will the launch vehicle's hardware have on biological cleanliness of the spacecraft? Most of the planning and attention goes into ensuring the spacecraft is assembled and tested in a clean fashion, but the launch environment should also be managed, or at a very minimum assessed.

In past missions, the launch vehicle hardware and environmental control systems were bioburden controlled and managed to understand their bioburden contributions as parts of the launch recontamination particulate modeling.[54,55,56] If the hardware is not managed, then manufacturing specification values found in Tables C-1 and C-2 in NASA-STD-8719.27 may be applied and used in particulate modeling. Upon identification of the potential bioburden contamination, a particulate recontamination analysis can be performed. While it is up to the project to determine the degree of modeling, this could vary from being a simplified particulate model assuming physical particle parameters to a more complex model that considers biological adhesion, surface roughness, and validated adhesion and removal models.[57,58]

[54]Benardini, James N, Myron T La, David Ballou, and Robert Koukol. 2014. "Implementing Planetary Protection on the Atlas v Fairing and Ground Systems Used to Launch the Mars Science Laboratory." Astrobiology 14 (1): 33–41. **https://doi.org/10.1089/ast.2013.1011**.

[55]Hendrickson, Ryan, Gayane Kazarians, Sarah Yearicks, Lisa Guan, Arman Seuylemezian, Linda Lee Matthias, Terry Schrepel, and James N Benardini. 2020. "Planetary Protection Implementation of the InSight Mission Launch Vehicle and Associated Ground Support Hardware." Astrobiology 20 (10): 1158–67. **https://doi.org/10.1089/ast.2019.2099**.

[56]Cooper, Matthew M, Fei Chen, Lisa Guan, Akemi A Hinzer, Gayane Kazarians, Cynthia Ly, T. Brian Shirey, and Kristina Stott. 2023. "Planetary Protection Implementation and Verification Approach for the Mars 2020 Mission." Astrobiology 23 (8): 825–34. **https://doi.org/10.1089/ast.2022.0046**.

[57]Barengoltz, Jack B. 1989. "Particle Adhesion to Surfaces under Vacuum." Journal of Spacecraft and Rockets 26 (2): 103–8. **https://doi.org/10.2514/3.26039**.

[58]Shallcross, Gregory S, William A Hoey, John R Anderson, Carlos Soares, and Moogega Cooper. 2024. "Modeling Adhesion and Aerodynamic Removal of Particles and Spores from Substrates." Journal of Aerosol Science 176 (February): 106294–94. **https://doi.org/10.1016/j.jaerosci.2023.106294**.

Several other factors that may influence modeling parameters or hardware implementation trade space include:

- ***Aeroshell and aeroshell vents (if applicable).*** The sizing, vent path angles, and potential filtration material could also contribute as modeling parameters to determine the ingress of bioburden during launch redistribution. Notably, bioburden deposited on the exterior of the aeroshell or other surfaces that experience entry heating may undergo a microbial reduction credit accordingly.
- ***Biobarriers***. These may be used to prevent launch recontamination, as was the case for Viking.[59,60]
- ***Launch vehicle fairing acoustic material.*** Given the large surface area that is directly exposed to the spacecraft, the material and bioburden management strategy should be developed sooner rather than later. Past missions had to conduct blanket bake-outs, trailblazer activities, mission unique cleaning protocols, and mission unique packaging to ensure biological cleanliness.
- ***Environmental control system stabilization.*** Prior to connecting the environmental control system to the launch vehicle, consider a system blow-down/break-in to remove particulates and settled particles and verifying/certifying cleanliness for contamination control.

4.0.0 PP Laboratory Quality Management System

Navigation Summary: *Chapter 4 discusses the implementation of a quality management system for PP laboratories conducting bioburden assays. This chapter is applicable to laboratory management and personnel for Category III, IV, or V missions using a bioburden measurement approach.*

OSMA uses quality management systems to determine compliance with descriptions of intent.[61] Laboratory quality management tools are available commercially or institutionally to support a quality approach. Quality objectives and descriptions of intent allow a program to develop a responsive implementation approach to address objectives within the resource means available. It can be tailored to the scalability and/or complexity of the bioburden requirements being verified. Quality can be measured in many different ways, and for PP labs, these descriptions of intent can include specifications, sampling and laboratory procedures, and plans that describe how personnel or equipment must progress through various actions. The measure of quality may be in inspection results, process control data logs, assay procedures, laboratory documentation, or records of completed work sequences. OPP uses a quality-based approach, including data acquisition, processing, and end use, to verify PP requirement compliance over a project's lifecycle.

This chapter discusses approaches to Quality Assurance (QA) for PP laboratory activities associated with bioburden quantitation and assessments. Detailed discussion is provided for laboratory quality management systems, including laboratory quality plans and method validation. Each section provides an example structure and framework for laboratories through a system of well-defined checks and controls to support PP compliance requirements. NASA encourages standardization of analytical approaches between NASA and industry to support both NASA and NASA-partnered missions. The approaches presented in this document mirror standard industry approaches. References to industry standards are provided at the end of this chapter.

Data Quality Objectives

Data Quality Objectives (DQOs) are a systematic process used as a planning and decision-making tool to support the project's required data outcomes. The DQO determines if the data generated in the lab is sufficient in quality and quantity to serve as credible evidence for verification of requirements. The DQO process is critical as it implements a balanced, structured, and stepwise planning strategy to define and document appropriate analytical methods; resource requirements; and the quality, type, and quantity of data needed to meet PP requirements. DQOs define tolerable data risk levels using performance and acceptance criteria metrics to meet project requirements. Data acceptance and performance criteria define

[59]Patel, Nikunj, Zachary Dean, Yuki Salinas, Lori Shiraishi, and Laura Newlin. 2019. "A Ground Support Biobarrier (GSB) for Recontamination Prevention." Life Sciences in Space Research 23 (November): 22–30. **https://doi.org/10.1016/j.lssr.2019.02.002**.
[60]Bonitz, Robert G., Lori Shiraishi, Matthew Robinson, Raymond E. Arvidson, P. C. Chu, J. J. Wilson, K. R. Davis, et al. 2008. "NASA Mars 2007 Phoenix Lander Robotic Arm and Icy Soil Acquisition Device." Journal of Geophysical Research 113 (null). **https://doi.org/10.1029/2007je003030**.
[61]**https://sma.nasa.gov/sma-disciplines/quality/**

the overall level of acceptable uncertainty in data outcomes via the understanding of analytical parameters and methods. These include method performance requirements such as method efficiency determinations, upper and lower detections limits, interferences (false or negative positives), and spacecraft sampling, including the area and number of samples to support bioburden statistics.

Minimally, the DQO process addresses the following:

- **Statement of the analytical objective:** Goals and expected outcomes
- **Schedule:** Timetable of work and key milestones
- **Required information inputs:** Type of data and how the data is used to support anticipated technical outcome
- **Performance or acceptance criteria:** Key parameters and analytical tolerances associated with data acquisition
- **Data acquisition plan:** Include resources, type, and quantity of data
- **Data QA requirements:** Include quality parameters associated with data collection and evaluation methods
- **Data management:** Include data and quality records associated with the project
- **Data analysis:** How the data is evaluated against the performance of acceptance criteria
- **Risk management (contingency planning):** Procedure to follow if data does not meet the specific requirements for the anticipated outcome

Process Validation

OPP's QA focus is process validation over a project's lifetime via the generation and implementation of appropriate DQOs using analysis or laboratory methods. DQOs describe the process and rationale used for data collection requirements. This replaces a previous approach heavily biased on auditing the mechanics of data acquisition rather than focusing on resulting data and data outcomes. In the past, validation of the assay process used comparative data from independent samples collected by NASA with those collected by the project. This involved coordinating multiple visits to flight cleanrooms throughout the integration, test, and launch operations; acquiring samples in parallel with project PP personnel; and shipping the samples to an independent laboratory for processing. The new verification strategy shifts from that comparative direct assay approach validating hardware bioburden at a "moment in time" to one that assesses the process of how PP data is acquired, laboratory and cleanroom management, and documentation in addition to a lower level of independent assay verification. This new approach to verification through independent assessment is described in more detail in *Section 4.2.0 Oversight and Concurrence Activities*. Projects should have a continual QA demonstration of the analytical process via project-established data requirements, laboratory management, and operational parameters throughout the mission's entire assembly process. Operational parameters could include things like routine equipment maintenance, sample control data trends, calibration schedule, training, etc. Laboratory operational parameters are evaluated against established QA requirements, shifting the validation of the PP data acquisition process to the project and its associated organizations. This transfers the demonstration of compliance from NASA to the project. OPP evaluates concurrence with project compliance against final demonstrated data outcomes.

"The process validation approach involves the systematic definition and management of processes, and their interactions, so as to achieve the intended results in accordance with the quality policy and strategic direction of the organization."[62] Laboratory quality-based systems and laboratory strategies are standardized and used across industry. PP demonstration of compliance includes implementing laboratory management systems, data quality and assessment strategies, monitoring, and routine validation (see Figure 17).

PP process validation has the following components:

- Process validation via the generation of appropriate DQOs, process implementation, and data capture that supports those objectives
- Laboratory compliance with analytical DQOs via standardized laboratory management and procedural requirements
- Demonstration of compliance throughout the process via independent internal assessment
- Implementation of a risk-based assessment program that accounts for the criticality of the mission, systems, subsystems, components, subassemblies, parts and materials, and the likelihood and severity of consequences in the event that compliance is not achieved

[62] "AS9100D: Quality Management Systems - Requirements for Aviation, Space, and Defense Organizations - SAE International." n.d. ww.sae.org. **https://www.sae.org/standards/content/as9100d/**. Page 7.

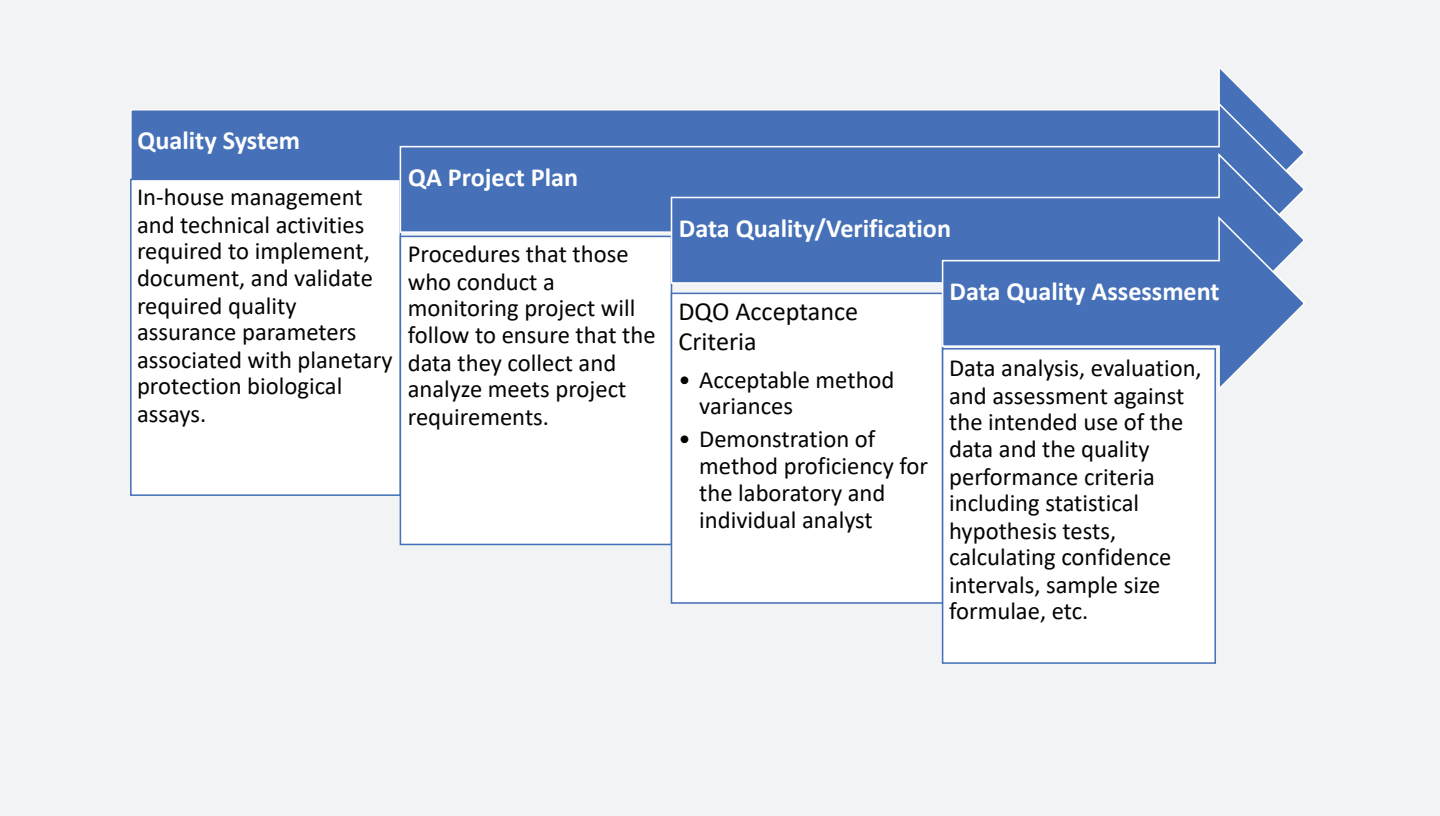

Figure 17: *A Performance-Based Validation Flow Shows the Quality System*[63]

Data Performance and Acceptance Criteria

Data performance and acceptance criteria are qualitative and/or quantitative statements of the overall level of uncertainty that is acceptable for the performance of biological assays to meet PP requirements. A project develops these criteria to support verification and validation of project bioassay requirements. The performance criteria describe the required study goals, data requirements and acquisition, and study boundaries to verify the data captured is of sufficient quality and quantity to meet the specified technical goals relative to the data's intended use. Data acceptance criteria for the project data use includes the trueness of the sample (i.e., does the sample directly represent flight hardware or is it a proxy), statistical validation of the sample set vs. surface area, and sample collection times (early assembly, final assembly).

Quality Management System

Routine quality assessments that verify the project's approach and data meets the end-use requirements are important criteria in a performance-based system. These assessments are implemented using a data quality system. A quality management system describes organizational responsibilities, processes, and procedures used to achieve PP goals. The system defines responsibilities and practices that are implemented to maintain the required data quality and is systematically evaluated for accuracy, consistency, completeness, timeliness, and validity. The data quality system includes a laboratory management system, systematic data acquisition planning, data quality validation and verification approaches, and a quality assessment of all data generated under the data quality system.

Laboratory QA Plan

The Laboratory QA Plan details the requirements to implement and manage a comprehensive laboratory quality system. This plan, in sync with organizational policies and procedures, outlines roles and responsibilities and management authority over the

[63]EPA Guidance on Systematic Planning Using the Data Quality Objectives Process, EPA/240/B-06/001 February 2006

laboratory and its activities. The plan details sublevel technical activities required to implement, document, and validate required QA parameters that are critical to maintaining an appropriate quality system to support associated PP biological assays and required project DQOs. The flowchart below provides an example of a laboratory quality system. The plan details the implementation of this system within the organization or project.

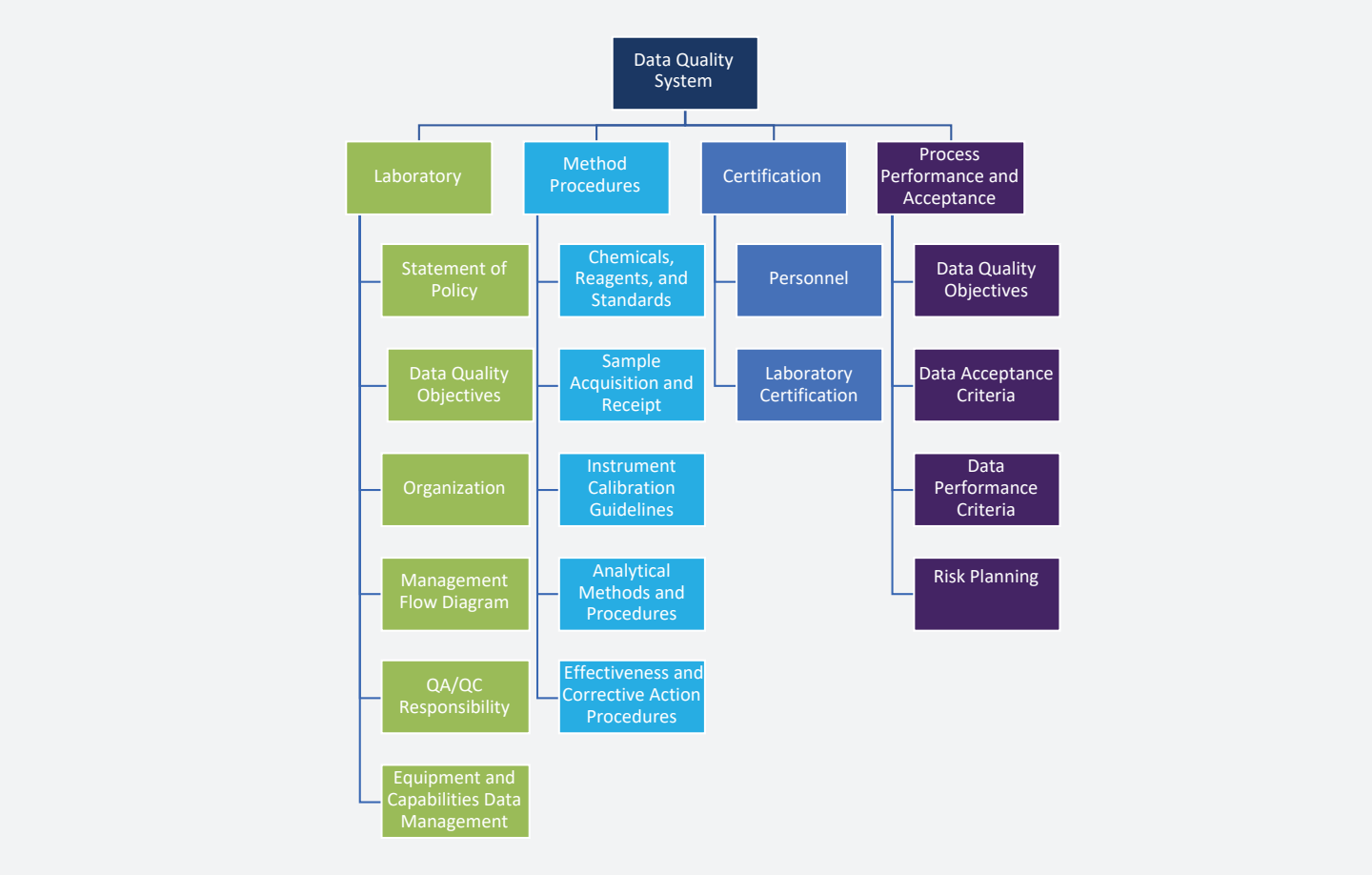

Figure 18: *General Topics Addressed by a Data Quality System*

The data quality plan should include the following topics:

Statement of Principles and Processes

A statement of guiding principles and processes details information on the components of the quality system and the processes used to implement the system. Areas of discussion include, but are not limited to:

- The laboratory process(es) on QA as it relates to its mission and the general goals of the policy, including resource allocation, personnel training, and funding.
- A description of the process(es) used to implement and assess the work performed.
- A description of the process(es) used to measure and evaluate QA activities.
- A description of the process(es) or structure that ensures effective communication of requirements, including systematic planning processes, PP implementation plans, QA plans, standard operating procedures, and technical assessments.
- A description of the laboratory facilities, including a diagram of the pertinent facilities and a list of critical instrumentation.

Summary of QA/QC Responsibilities

A successful QA program requires participation from all staff members; therefore, responsibilities for each staff member or organizational group should be clear and defined in the data quality plan. QA and Quality Control (QC) responsibilities are

addressed for the laboratory organization, which encompasses the organizational breakdown that defines QA responsibility at various staff levels to support laboratory principles and processes, including an outline of personnel and disciplines. QA oversight responsibilities are clearly defined, including those for day-to-day laboratory monitoring, data reporting requirements, corrective action, and recordkeeping and review. The independence of the data review process and sign-off levels of authority are also addressed and are implemented independently by personnel not directly involved with the work being performed.

Resources

Resource management is important to the success of the laboratory quality system. Detailed laboratory management and allocation for the proper QA resources are required to implement and maintain the quality system objectives. Resource allocation includes labor, training, travel, and equipment needed to perform the tasks and processes associated with the quality system.

Sample and Data Management Flow

Sample and data management flow strategies and procedures support the data quality process by ensuring that samples and data are handled in accordance with the data quality system requirements. Laboratory sample control and data management procedures address the traceability of sample acquisition, processing, and the data management process.

Sample and data traceability is a data management process that outlines a schematic queue of an individual sample from acquisition to sample disposal or archive, including data processing and review requirements to verify sampling and assay reliability and completeness in association with program requirements. The process of how acquired data from each sample is evaluated (review, validation, verification) and assessed against QA acceptance criteria is defined and documented. Sample rejection criteria is documented in the context of samples that do not meet sample acquisition or processing requirements.

Detailed laboratory requirements and provisions for sample handling should address sample storage/transport temperature, hold times, preservation, and other unique requirements noted in PP sampling standards. Tracking samples in the laboratory should be addressed, including appropriate temperature constraints and analysis schedules. Unique disposal or archiving requirements can be part of this procedure. Minimal recommended tracking parameters are listed below:

- Sample identification
- Sample date and time
- Identification of sampling personnel
- Sample location (building/room identifier)
- Specific location on hardware or GSE
- Sample type (wipe, swab)
- Surface area sampled (by wipe, swab)
- Sample matrix (material being sampled)
- Sample preservation (if required)
- Analysis requirements (NSA, molecular assay)
- Disposal or archive requirements

The laboratory data management process addresses standard recordkeeping procedures and document control, data storage and retrieval for raw and processed data, review mechanisms for detecting and correcting data errors, and the associated corrective action implementation and documentation requirements.

Effectiveness and Corrective Action Requirements

Each laboratory defines how the QA program is evaluated, the frequency of evaluation, and how the findings of the evaluation are assessed. The evaluation specifies how QA performance criteria are accessed via audits, technical assessments, and performance evaluations. The process addresses data review, validation, and verification, including a systematic approach to corrective actions when data fails to meet the prescribed DQOs.

QA Program Implementation Requirements

The QA plan details the laboratory implementation strategies, including all procedures and requirements to ensure full implementation of NASA PP requirements. Implementation approaches include required planning, implementing, and documenting quality assessment tools and the frequency with which they are used. The laboratory determines the level of competence, experience, and training needed to ensure that personnel performing assessments are technically knowledgeable and that laboratory personnel have required access to programs, managers, documents, and records to perform the evaluation. The laboratory plan defines how and when corrective actions are required, including the identification of root causes, the determination of whether the problem is unique or may have long-term implications, and a recommendation of procedures to prevent a reoccurrence.

Acquisition and Preparation of Chemicals, Reagents, and Standards

A sound quality program requires that laboratories control how supplies and consumables are acquired and accepted for project use. Acceptance criteria for each chemical, reagent, or standard should be determined based upon the DQO requirements and controlled to meet use and storage requirements, expiration deadlines, and other relevant requirements.

Standards are controlled similarly with a systematic procedure for verifying their validity and use. Any standard that fails to meet required validation criteria, exceeds the expiration date, or is compromised should be replaced.

General guidelines for chemicals, reagents, and standards are provided below:

- Standard reagents and other analytical supplies should meet basic lab standards set for the Laboratory QA Plan or as specified by specific analytical requirements, whichever is more stringent. In accordance with the laboratory specifications, supplies of known and required purity should be purchased from suppliers.
- Standards and reagents are prepared following specific method operating procedures by analysts certified in the method of interest.
- Laboratory water used in analysis is monitored to water quality requirements designated in analytical procedures. The method for monitoring and validating laboratory water quality is controlled and documented as required by the Laboratory QA Plan.

Instrument Calibration Guidelines

Instrument calibration procedures, documentation, and validation are included in the Laboratory QA Plan. Best practices and processes can typically be found in local/institutional policies (for example, NASA-STD-8739.12 and NASA center or other institutional policies on calibration). Any additional calibration requirements imposed to support PP laboratory analysis should be documented, including the frequency of calibration, procedures used for each instrument or tool, and documentation of each calibration event. Corrective action procedures are documented and implemented for instruments that are found to be out of calibration.

Analytical Methods Requirements

Each method performed in the laboratory should have designated QC procedures and requirements associated with each critical step. These requirements form a baseline for laboratory QA requirements. Methods and the associated developed QA procedures detail frequency, data acceptance criteria, and corrective action requirements during method implementation.

The following considerations should be addressed for each method:

- Quality check requirements including, but not limited to, field and laboratory blanks, duplicates, laboratory control samples, positive control samples, negative control samples, and calibration requirements
- Detailed calibration methods used for each method
- Detailed equations used for results calculations and to assess QA compliance
- Required control limits and corrective action requirements when such control limits are exceeded

Computer Hardware and Software

The management system for computer hardware and software details the process that ensures that computer hardware and software meet the required DQOs. This includes the validation of purchased hardware and software, the control process by which the laboratory validates in-house developed hardware and software, and software changes and updates. The system also addresses control processes for file management, data generation, and archiving.

Personnel

Each laboratory should have a documented plan for qualifying and training personnel to ensure that they have the required skills to effectively accomplish their work in PP. Analysts demonstrate competence through a laboratory training program, which includes the receipt of operating and analytical procedures, on-the-job training specific to each procedure, and a demonstrated ability to implement the procedures in the laboratory using control samples or other surrogate controls deemed appropriate for this purpose. The plan also documents training policies, processes, and roles and responsibilities.

A system documenting the training for each analyst should be in place to ensure that each analyst has the required knowledge, skill, and professional certifications or accreditations to perform in the laboratory, including a system for keeping personnel current as requirements, methods, and instrumentation change within the laboratory.

Operating Procedures

Each critical piece of equipment that is listed in the Laboratory QA Plan has a written procedure that defines the system's operation, use, and control requirements to verify that it is maintained and running properly.

Each analytical method should have a written procedure based upon PP documents and requirements as issued by NASA that defines detailed procedures and requirements to perform the method within the laboratory. Each method performed in the laboratory has designated QC procedures associated with each critical step. The procedure adequately reflects the quality implementation requirements within the laboratory structure including training requirements, QA, calculations, and corrective actions. Procedural updates or modifications require that all analysts who perform the method review and certify that the changes or modifications are understood and implemented by each analyst. Direct use of an OPP standard, such as the NASA Standard Assay found in NASA-STD-8719.27, may be substituted for an internal procedure.

The following considerations should be addressed within each protocol:

- Quality check requirements including, but not limited to, field and laboratory blanks, duplicates, laboratory control samples, positive control samples, negative control samples, and calibration requirements
- Detailed calibration methods used for each method
- Detailed equations used for results calculations and to assess QA compliance
- Required control limits and corrective action requirements when such control limits are exceeded

Methods

Prescriptive vs. Performance

QA can use both prescriptive and performance-based approaches. A prescriptive approach requires exact methods of quality assessment, including predetermined and specific analytical limits, metrics, and corrective actions if data quality acceptance requirements are not met. This approach is commonly used for Federal regulatory methods, such as those implemented by the Environmental Protection Agency (EPA) or the Food and Drug Administration (FDA), which require stringent data validation prior to data use. Performance-based approaches can use any form of quality assessment provided that the end use demonstrates an acceptable level of statistical control.[64] Figure 19 describes the two approaches.

[64] "15.4: Evaluating Quality Assurance Data." 2020. Chemistry LibreTexts. June 15, 2020. **https://chem.libretexts.org/Courses/BethuneCookman_University/B-CU%3A_CH-345_Quantitative_Analysis/Book%3A_Analytical_Chemistry_2.1_(Harvey)/15%3A_Quality_Assurance/15.04%3A_Evaluating_Quality_Assurance_Data**

Figure 19: *OPP Prescriptive, Performance-Based, and Blended Approaches Are Determined by the DQOs for the Project*

Laboratory Methods

Laboratory methods and analyses are used to assess and validate the cleanliness levels of spacecraft, assembly and launch areas, and cleanrooms. The current biological marker of interest is spore-forming bacteria, with work ongoing to assess and develop genomic-based methods. The development, use, or adaptation of analytical methods is determined by the DQOs required to meet PP goals. Analytical methods are developed and implemented using standard QA approaches. Analytical approaches fall into two categories: qualitative and quantitative.

Qualitative Data

Qualitative data is generally considered descriptive in nature, provides general knowledge capture, clarifies uncertainties, and informs the resolution process for a quantitative path forward regarding unknown hardware bioburden due to manufacturing, cleaning, or test processes. In most cases, the data will clarify and support the decision process to achieve quantitative or validated data for the parameter of interest.

Qualitative data may be used as part of the initial DQO process to help determine the analytical and data requirements needed to collect quantitative data used to support PP compliance. Qualitative data is used to characterize the technical concerns and approaches, define the type and quantity of data needed, and outline the analytical approaches and data outcomes required to resolve PP issues. Qualitative data collection requires appropriate quality controls to meet the data end-use requirements and may include some or all of the parameters addressed in the EPA's QA Parameters Section of their Guidance on Systematic Planning Using the Data Quality Objectives Process document.[65]

Quantitative Data

Quantitative data is numeric data collected using a structured system of precise measurements to support and confirm bioburden calculations associated with PP compliance requirements. Quantitative data statistically estimates and quantifies

[65] **EPA Guidance on Systematic Planning Using the Data Quality Objectives Process** provides a systematic approach for formulating DQOs.

data uncertainty, variability, reliability, and risk to determine if the generated numerical data supports the acceptance criteria, conclusions, and defensible outcomes required by the project's PP goals.

Analytical methods are the most common tools for providing quantitative data to support bioburden accounting, launch vehicle assessments, and recontamination estimates. Understanding an analytical method's performance is critical to meeting data acceptance criteria parameters and requires that the method's performance be fully vetted operationally. QA parameters for each method are performed, analyzed, and used to establish and maintain laboratory operational requirements for the method and the statistical basis for the end use of the data sets generated by analysis.

Method Efficiency

The analytical efficiency determination currently used in association with the NSA is a quantitative measurement that combines sample collection and the analytical method.[66] The efficiency is applied to the assay data to essentially "correct" the total spore values based upon the entire assay (sample acquisition and laboratory analysis) recovery. Efficiency determinations are essential to this process but are complex due to the nature of the assays that are conducted on flight hardware. To date, efficiencies used by projects for the spore assay have been statistically supported estimates supplied by OPP.[67] The efficiency validation, published or experimentally determined, now resides with the project's laboratory, either in-house or external, and should be determined based upon operational requirements set forth in the laboratory QA documentation. Once the efficiency is analytically determined, the QA plan should address when the efficiency determination should be reverified. Changes in personnel, sample tools, relevant laboratory equipment, laboratory set-up, or location changes all are indicators of a need to revalidate method efficiencies.

Sample Acquisition

There are several factors to consider in terms of sample acquisition.

Sample Tools

Sampling flight hardware or its associated GSE requires the removal and capture of spores or biological fragments on the sample tool surface. Sample tools do not have equivalent removal characteristics on hardware surfaces. In addition to meeting laboratory DQOs, the tools must be compatible and approved for use in cleanrooms and on spacecraft via the project/institution's requirements. The sampling tools are used to swab or wipe surfaces in accordance with the requirements of NPR 8715.24. Ideally, sample tools are chosen to maximize the removal of the constituent of interest from the surface with additional consideration for other flight requirements, such as contamination control and potential surface contamination during sample acquisition. The type of analysis will influence the sample tool choice based upon possible interferences with the analytical method requirements. For example, an assay conducted for spores that is analyzed by the NSA pour plate method has different requirements than samples collected to support a biochemical analysis, such as Adenosine Triphosphate (ATP). A sterile cotton swab that is appropriate and used to collect spores would contribute unwanted cellular material (as an interference) during the analytical workup for ATP. The type of sample tool should be addressed when developing the DQO associated with sample acquisition and data acceptance criteria strategies for the project.

Surfaces

The ability of a specific sample tool to reliably remove the constituent from the surface of interest is greatly complicated by the variety of surfaces associated with a flight project. More common surfaces include titanium, aluminum, multi-layer insulation, fabrics used for parachutes, and launch vehicle blankets, with the variability in surface structures greatly affecting a sample tool's ability to physically remove organisms such as spores. Surface properties may appear uniform to the eye, but most have microscopic surface textures such as pores, woven components, surface chemistries, and other characteristics that can entrap spores or make removal difficult, thereby affecting the overall efficiency of the collection system.

[66] NASA Standard Assay, **NPR 8715.24**
[67] **https://www.iapsam.org/PSAM16/paper.php?ID=AR41**

The Human Factor

OPP requires sample collection be performed by trained PP analysts. Sampling and the associated training protocols are generated as part of the laboratory QA documentation. Variances, even if slight, in individual sample collection techniques can affect final efficiency data. The effect that individual samplers may have on the method efficiency should be included as part of the overall method efficiency determination.

Method Validation

Method validation is a process that is used to demonstrate the suitability of an analytical method for an intended purpose. The validation process confirms performance characteristics associated with the method and demonstrates method limitations that should be considered during output data assessments. Validation includes the determination of the method's overall efficiency to support PP bioburden calculations. The validation process uses systematic analytical determinations of specific method outputs to determine the overall effectiveness of the method. The required parameters for method validation include:

1. **Accuracy:** Expressed as the percentage of recovery of microorganisms by the method
2. **Precision:** The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of homogeneous suspensions of microorganisms under prescribed conditions
3. **Specificity:** The ability to detect the required range of microorganisms that may be present in the sample under test (see Microbiological Strains below)
4. **Limit of Detection:** The lowest and highest numbers of microorganisms that can be accurately counted
5. **Robustness:** The measure of the procedure's capacity to remain unaffected by small but deliberate variations in method parameters; provides an indication of the method's reliability under a variety of normal test conditions, such as different analysts, instruments, batches of reagents, and laboratories
6. **Linearity:** The ability to produce results that are proportional to the concentration of microorganisms present in the sample within a given range in the sample

Microbiological Strains

Previously accepted challenge organisms to demonstrate the pour plate method's specificity include:

- *Bacillus atrophaeus ATCC 9372*
- *Bacillus safensis DSMZ 19292*
- *Bacillus megaterium DSMZ 32*
- *Bacillus megaterium 2c1*
- *Bacillus thuringiensis E24*
- *Bacillus thuringiensis DSMZ 2046*

Organisms that are included in this test work should be from sources that are certified or reliable culture collections.

Method Verification

Operational changes during the use of the method over a project's lifetime may affect the method's performance. Changes may include, but are not limited to, new personnel, new reagents or equipment, new sample tools, and laboratory moves from assembly areas to launch or onsite laboratories. Once the method is validated, the QA system requires that the method be periodically verified as needed to ensure that it is performing as intended over the project's lifetime. Verification may not require a complete set of validation tests other than examining the relevant parameters to address method performance concerns. The verification criteria should be addressed in the method documentation.

Risk Management

NPR 8000.4C Agency Risk Management Procedural Requirements details NASA's risk management approaches. NPR 8715.24 outlines OPP risk responsibilities. The project QA system addresses organizationally and specifically the implementation of a risk-based assessment program that accounts for the criticality of the systems, subsystems, components, subassemblies, parts, and materials, and the likelihood and severity of consequences if PP requirements are not achieved.

Summary

OSMA uses quality to measure the collection, evaluation, and use of data generated to meet NASA PP requirements. Projects and organizations respond to PP requirements by implementing a QA system to manage and control required data outcomes via ongoing QA and QC activities. The QA system provides a process for planning, implementing, and assessing PP data using standard QA approaches, such as DQOs, analytical method validation and verification, laboratory QC parameters, and metrics. Project and laboratory personnel are trained to support and contribute to the quality process, which continually monitors and evaluates data quality throughout the mission's entire assembly process.

OPP uses a continual quality process and its associated outputs as a basis for data evaluation, risk evaluation, and concurrence of project compliance.

Useful References

NASA

- **NPR 8715.24 Planetary Protection Provisions for Robotic Extraterrestrial Missions**
- **NPR 8735.2C Hardware Quality Assurance Program Requirements for Programs and Projects (Updated w/Change 2)**
- **NPR 8000.4C Agency Risk Management Procedural Requirements**
- **NASA Standard 8719.27 Implementing Planetary Protection Requirements for Space Flight**

EPA

- **EPA Guidance on Systematic Planning Using the Data Quality Objectives Process, EPA/240/B-06/001 February 2006**
- **EPA Guidance for Quality Assurance Project Plans AQ/G-5, EPA/240/3-02/009 December 2002**
- **EPA Quality Manual for Environmental Programs, CIO 2105-P-01-0, May 5, 2000**
- **EPA Microbiological Alternate Test Procedure Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods, EPA 821-B-03-004, July 2003**
- **EPA Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples, October 2004**
- **EPA Overview of the EPA Quality System for Environmental Data and Technology, EPA/240/R-02/003, November 2002**

FDA

- **Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, Edition 3.0, U.S. Food and Drug Administration Foods Program, October 2019**
- **Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products, Edition 1.2, U.S. Food and Drug Administration Foods Program, December 2023**

Other

- **ISO/IEC 17025:(2017) General Requirements for Competence of Testing and Calibration Laboratories**
- **Poppiti, J. Environ. Sci. Technol. 1994, 28, 151A–152A**
- Evaluating Quality Assurance Data: David Harvey, **15.4: Evaluating Quality Assurance Data – Chemistry LibreTexts**
- European Pharmacopoeia, **5.1.6 Alternative Methods for Control of Microbiology Quality**
- **World Health Organization, Clinical and Laboratory Standards Institute, Supplement To The Laboratory Quality Management System Training Toolkit, Module 16 – Documents and records Quality manual version 2013**
- United States Pharmacopeia, **Chapter 1225 Validation Of Compendial Procedures, August 1, 2017**
- United States Pharmacopeia, **Chapter 1226 Verification Of Compendial Procedures, December 1, 2019**
- **SAE Aerospace Standard AS9100 Rev D, Quality Management Systems – Requirements for Aviation, Space, and Defense Organizations**

4.1.0 Proficiency Testing

As part of the QA approach for flight projects, there should be a reasonable assurance that analysts are properly qualified and trained to perform assays. In general, laboratory proficiency testing (PT) is an independent quality and accuracy assessment that tests qualified and controlled samples from an outside source to ensure accurate lab testing results. PT is often an integral part of a laboratory's QA program.

Industry and Government agencies including the FDA, EPA, and the American Industrial Hygiene Association (AIHA) use PT as part of their laboratory validation criteria. PT provides a comparative and objective assessment of a lab staff's competencies, sample handling, equipment functionality, and results reporting. Inter- and intra-laboratory testing can be used to demonstrate consistency, and the results can be used to monitor and improve the lab's quality of measurement. Information obtained from PT can help identify potential problems before they happen and lead to better resolutions. "A proficiency testing program is one of many QA measures that allow the laboratory to demonstrate its ability to:

- Confirm continued competent performance
- Compare performance with other laboratories
- Compare performance amongst analytical staff
- Identify areas for improvement, and
- Ensure customer confidence in the reliability of the laboratory's work product."[68]

Many industrial and Government regulatory agencies require that laboratories seek accreditation to validate in-house sampling, and testing procedures are performed with adequate controls by well-qualified personnel using appropriate equipment and methods. For example, independent programs certified by the ASTM or the AIHA are statistical QA programs that enable laboratories to assess their performance via comparison with other laboratories in the same program. Standard industry practices include an independent proficiency assessment or comprehensive internal PT programs performed at regular intervals.

Certified Reference Materials (CRMs) are highly characterized and controlled standards/samples that may be used to evaluate a laboratory's performance under specified conditions relative to a given set of criteria in a specific area of testing. In theory, CRMs can be used to:

1. Demonstrate and document analyst proficiency
2. Evaluate initial performance of new analysts
3. Evaluate repeatability between two or more analysts
4. Evaluate the performance between two or more different laboratories against known values

PT Programs

External PT programs are initiated from and reported to a qualified provider external to the laboratory's QA program. External PT includes a performance rating, preferably a proficient or non-proficient rating based on a common statistic or other procedures acceptable within the program's criteria. Participants are required to meet the statistical performance requirements or perform CAs and retest to maintain program compliance.

The AIHA recommends the following parameters for an internal proficiency test, which is initiated, reported, and validated via the laboratory's QA system:

- A minimum of 20 QC data points obtained initially to determine upper and lower control limits at 3 standard deviations.
- The laboratory implements the PT challenge at least twice per year, with each round separated by approximately six months or when laboratory control requirements change or are not being met during analysis. It is recommended that the time between PT rounds for each analyst be a minimum of 15 days apart.

[68]Scientific Working Group for Forensic Anthropology (SWGANTH) Proficiency Testing Issue date: 08/02/2012 Revision: 0 **https://www.nist.gov/system/files/documents/2018/03/13/swganth_proficiency_testing.pdf**

- Each round consists of a minimum of four independently prepared blind spikes or standards at varying levels with the resulting data evaluated against the laboratory's pass/fail criteria demonstrated and documented during the laboratory's method validation. Samples are analyzed via the normal procedure. All colonies are to be counted and Too Numerous to Count (TNC) results in a "no evaluation" standing for the sample. The laboratory's management system documentation fully describes the standard/spiking levels, frequency, responsibility for PT implementation, statistical treatment of resultant data, acceptance criteria, and actions to be taken in the event of an unacceptable result. The spiking must be performed on an appropriate matrix that mirrors samples processed using the method in routine analysis.[69]

Both external and internal PT programs require corrective actions if the data indicates a "not proficient" standing for the analyst. The PT corrective action criteria is defined in the laboratory's QA system and includes, but is not limited to, identification and resolution of the root cause of "not acceptable" results to continue participation in method analysis and participation in supplemental or additional testing to satisfy PT requirements before returning to analysis.

4.2.0 Oversight and Concurrence Activities

While it is the continued responsibility of the mission to provide DQOs and subjective evidence to demonstrate that PP requirements are met throughout the lifecycle of the mission, as the TA, OSMA will oversee these activities through independent oversight to develop a substantiated concurrence approach. Tracking the project's execution of planned implementation throughout the project lifecycle involves a series of activities that demonstrate that the mission's documented compliance and data quality meet PP objectives. OSMA assesses the success of overall PP process implementation and the resulting reported data throughout the project lifecycle. The assessments and related activities are arranged with coordination and agreement between OSMA, OPP, center SMA, and the mission.

Examples of oversight activities that may be leveraged by OSMA and how they may be implemented on a project could include, but are not limited to, the following:

- **Independent assessments of the process.** An independent assessment of the PP processes implemented on a mission may be requested to provide visibility during the project's lifecycle. The assessment evaluates the mission's PP processes and provides quantitative and qualitative data-driven information to support the data quality requirements.

  For these assessments, the OPP requests that the NASA Safety Center's (NSC) Independent Assessment and Audit Team perform an assessment on the center and/or mission's PP process within a given mission. The processes may be general to overall requirements or mission-specific as relevant to the PP requirements. The purpose of these assessments is to ensure effectiveness of biological contamination control and the validity of data generated to support PP biological cleanliness assessments and required reporting. The assessment can be used to evaluate processes, procedures, analyses, laboratory methods and materials, and facilities in use to implement PP.

  The NSC's audit team may conduct documentation reviews, virtual interview sessions, on-site visits, and on-site interviews to further understand the process flow and observe measures and mitigation being implemented to ensure cleanliness. After the assessment observations are completed, the assessment team will write a report and brief OSMA, center SMA leadership, and mission management. The findings and recommendations from the assessment will then be further discussed with OSMA with the key focus of improving the practices, policies, and procedures within the discipline.

- **Independent assays of spacecraft-associated environments, facilities, and flight hardware.** For these assays, the oversight authority and OPP works with the mission to identify mandatory PP inspection points for conducting an assay. The OPP and mission identify access and surfaces to be sampled. The project then collects the samples and ships them to an independent testing laboratory for analysis by the OPP team. During the sampling event, the OPP may have a representative on-site or virtually to witness the sampling event. Results from the independent analysis are then compared with the mission data to verify a mission's reported cleanliness levels at the time of sample collection. Independent assays may also be used to assess PP anomaly mitigation strategies used by the project. Depending on the mission and hardware flow, there may be multiple independent verification assays throughout the lifecycle of the mission.

[69] AIHA Laboratory Accreditation Programs, LLC AIHA-LAP, LLC Policy Module 6 Effective Date: October 3, 2016 Revised: August 3, 2016 Revision: 4. **https://www.aihaaccreditedlabs.org/policies**

- **Inspection and assessment of spacecraft-associated environments, facilities, and microbiological labs.** For these inspections and assessments, OSMA will work with the mission team to identify key environments, facilities, or microbiological labs to inspect and assess. This is a focused effort on a particular process and environment used to support PP compliance. Inspections can occur as part of a nominal oversight function or during an anomaly situation and may include a review of the documentation and quality practices as well as on-site visits. After the inspections and assessment observations are completed, the inspection team will write up a report and brief OSMA and mission management. The observations and recommendations from the assessment will then be further discussed with OSMA with the key focus of improving the practices, policies, and procedures within the discipline.
- **Concurrence of identified requirements, implementation plans, and reports.** Obtaining concurrence for PP compliance requires clear documentation and traceability to NASA and international consensus standards. Sound technical rationale and subjective evidence must be provided to substantiate concurrence in a clear and logical flow. Engineering parameters and implementation should have appropriate rationale and data to substantiate implementation and data outcomes. Deviations or anomalies should have a detailed technical rationale for why the intent of the requirement or standard was still upheld.

5.0.0 Biologically Controlled Cleanrooms

Navigation Summary: *Chapter 5 discusses best practices for managing cleanrooms for PP missions. This chapter is applicable to any mission being built in a cleanroom, but especially for Category III, IV, and V missions and PP laboratories controlling/monitoring cleanroom bioburden as part of their implementation approach.*

Cleanroom, Biologically Controlled: *Room within which the facility procedures and operations are designed for biological contamination prevention and awareness, including cleaning and monitoring for levels of biological contamination by use of full body coverall, hood, face mask, gloves and boots, and restricted access.* (NASA-STD-8719.27 definition)

Biologically controlled cleanrooms need to meet the particle limits defined in ***ISO 14644-1*** as well as utilize multiple strategies to control and measure bioburden as described below. Management of a biologically controlled cleanroom should be detailed in the Planetary Protection Implementation Plan (PPIP), and data from the cleanroom monitoring maintained with project records. Specific bioburden action and alert levels should be established along with corrective action and documentation plans.

ISO 14644-1:2015 describes particle-controlled cleanroom particle limits and the certification process. Planetary flight hardware is generally integrated in ISO Class 8, 7, or 5 cleanrooms, depending on the needs of the mission. Verification and monitoring of cleanroom particulate levels is often managed by facilities or contamination control teams, and should include both room level verification scheduled to maintain certification as well as continuous monitoring of particles close to sensitive hardware.

Tables C-1 and C-2 in NASA-STD-8719.27 describe manufacturing specification values that serve as baseline bioburden levels that can be applied in various manufacturing environments. These levels are based on the ISO cleanroom class of the facility and whether additional controls are in place to control bioburden. In certain situations, these values can be applied to hardware that cannot or will not be directly tested for bioburden. When specification values are applied, a mission should be able to justify why those values are appropriate to their manufacturing process and demonstrate that cleanliness is maintained when hardware moves between clean environments as well as during shipping and launch operations.

Cleaning with Disinfectants

A biologically controlled cleanroom should be frequently cleaned using multiple disinfectants with different mechanisms of biological inactivation in rotation to eliminate organisms that are resistant to a single cleaning method. In some instances, cleanroom microorganisms were found capable of metabolizing the chemicals in cleaning products.[70] Activity against bacterial endospores should be considered as these are resistant to many chemical treatments. The Centers for Disease Control and

[70]Mogul, Rakesh, Gregory A. Barding, Sidharth Lalla, Sooji Lee, Steve Madrid, Ryan Baki, Mahjabeen Ahmed, et al. 2018. "Metabolism and Biodegradation of Spacecraft Cleaning Reagents by Strains of Spacecraft-Associated *Acinetobacter*." *Astrobiology* 18 (12): 1517–27.

Prevention's (CDC's) **"Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008 Update: May 2019"** details mechanisms of many of the common disinfectant treatments. When choosing disinfectants, consider the mechanism of action, compatibility with cleanroom and hardware materials, risk of residue (both from the disinfectant itself and any impurities in the formulation), and any risks posed by chemical odor or vaporized chemical. If disinfectants used leave a residue that could interfere with downstream bioburden accounting, then appropriate neutralizers should be added to media used to process samples.

Some common disinfectants used in planetary mission cleanrooms include isopropanol (70-99%), ethanol (70-99%), hydrogen peroxide (3-6%), a mixture of peracetic acid and hydrogen peroxide, phenolics, and detergents. The PPIP should detail the frequency of cleaning along with disinfectants and cleaning agents to be used.

Bioburden Monitoring

While there are no biological cleanliness requirements for facility surfaces, GSE, or facility air in NASA-STD-8719.27, it is highly encouraged for missions to assess the risks for spacecraft recontamination from the facility and establish mission-specific requirements and a facility monitoring plan. Various Mars rover and lander missions at NASA have implemented this plan.[71,72,73,74,75,76,77] Missions may also consider industry standards to help set biological thresholds in cleanroom environments.[78,79,80] The mission may consider biological cleanliness industrial standards such as ISO 14698-1:2003, which provides direction on facility monitoring, personnel garmenting recommendations, and cleanroom practices and protocols to help reduce biological contamination in the cleanroom environment.[81] The monitoring plan should be detailed to include frequency and quantity of routine surface and air sampling for viable microorganisms and may include rapid bioassays or sampling for metagenomic analysis as required by the mission. The PPIP should also include details on how cleanroom surfaces, GSE, and flight hardware are cleaned, verified, and monitored for bioburden levels. Guidance on sampling and sample processing can be found in *Chapter 6.0.0 NASA Standard Assay Laboratory Considerations*. Action and alert levels should be established and will depend on the stringency of the room and/or the sensitivity of the hardware. Corrective actions, including re-cleaning or changing cleaning frequency, will need to be implemented if monitoring detects high microbial levels.

Important elements of a sampling plan can include but are not limited to sampling locations, frequency of sampling, size of samples, types of samples, time of day, facility activity level, and type of sample processing and are described in more detail in ISO 14698 Section 5.3.2.4,[82] IEST-RP-CC023.2 Section 9,[83] and ECSS-Q-ST-70-58C Section 5.[84] Factors to consider when developing a cleanroom monitoring plan include the activity level of the cleanroom, frequency of hardware moving in and out, airflow configuration, and cleanliness requirements. Monitoring for particulate and molecular contamination should also be established but may be described in the mission contamination control plan and referenced by the PPIP.

Ultraviolet Germicidal Radiation

Ultraviolet-C light (UV-C), 180-280 nm, can be used to decrease viable bioburden in air or on surfaces in a cleanroom or laminar flow workbenches. Various lamp sources and wavelengths were studied and shown to be effective at inactivating a range of microorganisms. At the appropriate intensity and exposure time, UV-C has been shown to inactivate many types

[71] Benardini, J. N., La Duc, M. T., Beaudet, R. A., & Koukol, R. (2014). "Implementing planetary protection measures on the Mars Science Laboratory." *Astrobiology*, 14(1), 27–32.
[72] Benardini, J. N., La Duc, M. T., Ballou, D., & Koukol, R. (2014). "Implementing planetary protection on the Atlas V fairing and ground systems used to launch the Mars Science Laboratory." *Astrobiology*, 14(1), 33–41.
[73] Hendrickson, R., Kazarians, G., & Benardini, J. N. (2020). "Planetary Protection Implementation on the Interior Exploration Using Seismic Investigations, Geodesy and Heat Transport Mission." *Astrobiology*, 20(10), 1151–1157.
[74] Cooper, M., Chen, F., Guan, L., Hinzer, A. A., Kazarians, G., Ly, C., Shirey, T. B., & Stott, K. (2023). "Planetary Protection Implementation and Verification Approach for the Mars 2020 Mission." *Astrobiology*, 23(8), 825–834.
[75] See footnote 73.
[76] See footnote 74.
[77] See footnote 75.
[78] **US FDA. September 2004 Pharmaceutical CGMPs. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice.**
[79] **World Health Organization WHO Technical Report Series, No. 961, 2011 Annex 6. WHO good manufacturing practices for sterile pharmaceutical products.**
[80] **ECSS-Q-ST-70-55C 15 November 2008. Space product assurance - Microbial examination of flight hardware and cleanrooms.**
[81] **ISO 14698-1:2003. Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods.**
[82] International Organisation for Standardisation, "ISO 14644-1 Classification of air cleanliness by particle concentration," *International Standard: Cleanrooms and associated controlled environments*, 2015.
[83] "IEST-RP-CC023: Microorganisms in Cleanrooms." n.d. Www.iest.org. Accessed February 6, 2024. **https://www.iest.org/Standards-RPs/Recommended-Practices/IEST-RP-CC023**
[84] "ECSS-Q-ST-70-58C – Bioburden Control of Cleanrooms (15 November 2008) | European Cooperation for Space Standardization." n.d. Ecss.nl. Accessed February 6, 2024. **https://ecss.nl/standard/ecss-q-st-70-58c-bioburden-control-of-cleanrooms/**

of microorganisms, leading to a multi-log microbial reduction.[85] Project-specific verification and validation can support further microbial reduction. The effectiveness of UV-C exposure is dependent on several factors, including intensity of the light source, distance from the surface, and time of exposure. It is also best used for line-of-sight applications. It is recommended that the intensity of the UV-C radiation be measured at the furthest exposed surface to determine how long the exposure should be.

UV-C Safety

Exposure to ultraviolet (UV) light can be hazardous to humans, so the use of a germicidal UV system should be approved by institutional safety personnel to prevent exposure. It is suggested that any UV-C system be used only when a space is unoccupied, along with an interlock system to prevent accidental exposure. UV-C exposure can also damage or cause bleaching of some materials, as well as potentially generate undesirable side effects. Compatibility with cleanroom and hardware materials should be considered. Depending on the type of bulb used, UV light can generate ozone, so levels of ozone and air handling should be included in safety considerations.

5.1.0 Cleanroom Entry

Cleanroom contaminants can come from exterior sources as people, flight hardware, and GSE move in and out of cleanrooms, or they can be generated by activities in the cleanroom. This section covers considerations for ensuring items entering the cleanroom do so in a way that maintains PP cleanliness requirements.

5.1.1 Personnel Entry

Personnel

The primary source of contamination that finds its way into the cleanroom environment originates from the humans who work there. People naturally shed particles, including skin cells, hair, and respiratory droplets. Our clothing also sheds as we move around and experience airflow in the room. As a result, cleanrooms require special dress considerations, known as cleanroom gowning. Gowning is critical to maintaining a clean hardware assembly environment as it serves as a means to contain a large portion of human and clothing-based particles.

For spacecraft with missions that target sensitive planetary bodies such as Mars or icy moons in the outer solar system, most hardware is assembled in cleanrooms that require workers to wear full cleanroom garments, colloquially known as “bunny suits.” A bunny suit is a garment worn over street clothes to prevent contaminants such as clothing particles, hair, and skin cells from entering the cleanroom. The full bunny suit gear includes single use items such as shoe covers, booties, hair covers, face masks, and gloves, along with reusable (washable) components such as a hood to cover the neck and hair, jumpsuit, and shoe covers. Workers wear bunny suits at all times in the cleanroom environment. Most suits are made from non-particle generating, anti-static fabric, and workers must change their suits frequently to maintain optimum cleanliness levels. The garments are laundered to project or facility-specified levels on a regular schedule. Many projects also require tape to secure gloves to cleanroom garment sleeves to prevent slippage which could expose a wrist or bare arm during cleanroom activities. Gloves are either replaced, multiple layers used, or solvent cleaned throughout the assembly process to minimize direct transfer of particulates around the cleanroom. All gowning and de-gowning takes place in the change room. There are special procedures for gowning and gown removal when entering or exiting the cleanroom.

Personnel who work in the cleanrooms receive specialized training and certifications on how to suit up. The process of gowning starts upon entering the garmenting change room by applying shoe covers, a beard/face cover or mask, and a disposable hair cover. Next is a cleanroom hood cover over the disposable hair cover. It is crucial to take care to keep the bunny suit from touching the floor during gowning and minimize contact between the exterior of the garment and personnel skin/clothing as much as possible. The bunny suit is then carefully pulled over the legs, the arms inserted in the sleeves, and the head cover

[85]Masjoudi, Mahsa, Madjid Mohseni, and James R. Bolton. 2021. “Sensitivity of Bacteria, Protozoa, Viruses, and Other Microorganisms to Ultraviolet Radiation.” *Journal of Research of the National Institute of Standards and Technology* 126.

tucked into the neck before zipping and closing the suit. Then, boots are put on and adjusted for a snug fit. Gloves are put on and taped at the wrist. Before entering the clean area, an air shower is used to remove loose particulate or fibers from the exterior of the cleanroom garment. Upon leaving the cleanroom, the worker should immediately return to the change room to remove the cleanroom suit. Getting unsuited reverses the gowning procedure. Special care is taken to ensure that gowns intended to be reused do not become contaminated during storage. Suits on hangers should be stored such that the interior surfaces do not touch the exterior surfaces of the same suit, or the suit on the next hanger over. Shoes should never be stored above gowns. Gloves are kept on during the de-gowning and storage of suits.

While working, individuals cannot pull down their face mask or remove any part of the cleanroom gear. A second pair of gloves is put over the first pair upon entry to the cleanroom. This second pair can be changed out as needed; if someone touches the floor with their glove or takes a phone call, the top set of gloves can be replaced.

Bunny suits are essential to maintain biological cleanliness for many cleanroom purposes, including PP. Many industries use cleanroom suits, including medical suppliers and the semiconductor industry. For PP, suits and gloves provide a barrier between humans that build spacecraft and the flight hardware.

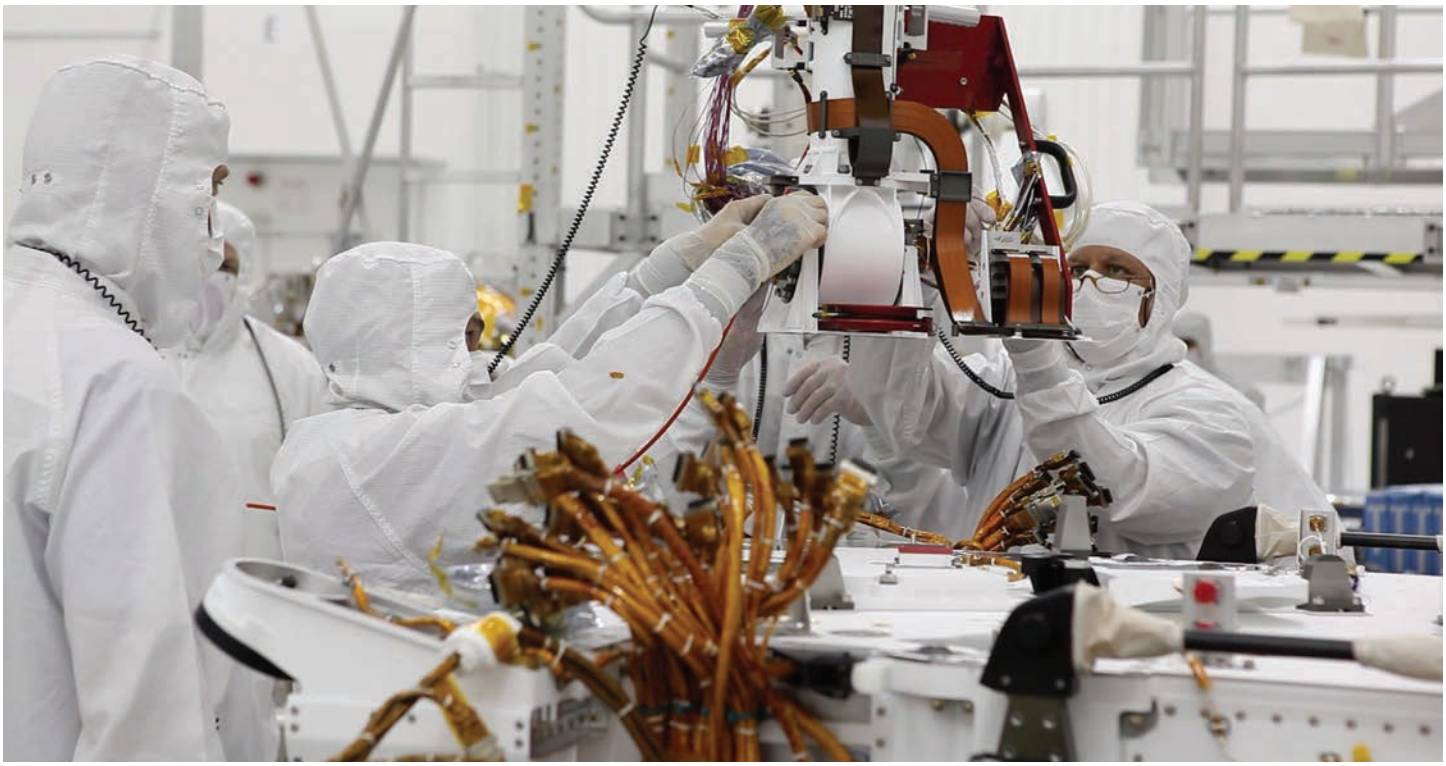

Figure 20: *Engineers at NASA's Jet Propulsion Laboratory are dressed head to toe in bunny suits, and only their eyes and foreheads can be seen. Photo credit: NASA*

In addition to gowning, other protocols in cleanrooms ensure the hardware stays as clean as possible. Although a cleanroom can be busy, the number of people allowed in the room at any one time is controlled and monitored. During cleanroom operations, everyone should be aware of their physical actions and activities and mindful of any actions that might compromise cleanliness requirements in the cleanroom.

Considerations for maintaining cleanroom cleanliness and PP requirements with entry and egress include:

- Verification before entry
- Frequency of entry and egress
- Passthrough rules/operation
- Large items entering (no airlock) pressure maintenance
- Timeline for verification of items or reverification of cleanroom

Additional Resources

- "ISO - ISO 14698-1:2003 - Cleanrooms and associated controlled environments — Biocontamination control — Part 1: General principles and methods." **https://www.iso.org/standard/25015.html** (accessed Feb. 10, 2023).
- "Standard Guide for Evaluation of Cleanroom Disinfectants." **https://www.astm.org/e2614-15.html** (accessed Feb. 10, 2023).
- NASA OSMA, "NASA-STD-8719.27 Implementing Planetary Protection Requirements for Space Flight." NASA, Aug. 22, 2022. Accessed: Jan. 12, 2023. [Online]. Available: **https://standards.nasa.gov/sites/default/files/standards/NASA/Baseline/0/NASA-STD-871927-Baseline.pdf**
- **W. A. Rutala and D. J. Weber, "Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008."**
- W. Kowalski, "Ultraviolet germicidal irradiation handbook: UVGI for air and surface disinfection," *Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection*, pp. 1–501, 2009, doi: 10.1007/978-3-642-01999-9/COVER

5.1.2 GSE Cleaning and Entry

GSE is critical to spacecraft build procedures. The equipment includes hardware rotation fixtures, cranes, hardware used in specialized lifts and moves, shipping crates, and tall ballymores and ladders. These types of GSE are complex and extremely large. GSE also includes electrical cables, tools, vacuum hoses, computers, phones, tables, benches, chairs, and any other non-flight equipment used to support the assembly activities. Cleanliness and bioburden requirements for GSE should be established and documented in the PPEL. These requirements are usually based on the sensitivity of the mission and the risk that particular GSE could contaminate the hardware (typically because of contact with or proximity to flight hardware).

Figure 21: *This image shows major components of NASA's Mars 2020 Mission in the High Bay 1 cleanroom in the Jet Propulsion Laboratory's (JPL's) Spacecraft Assembly Facility. The spacecraft is in the center of the photo. Note all of the GSE in the area to support assembly activities. Photo credit: NASA*

Good cleanroom practices are required for these items, including multiple cleaning requirements and GSE bioassays to make sure items are clean before using them in the hardware's vicinity. Most GSE is cleaned and assayed before it enters the cleanroom.

Large equipment provides unique cleaning challenges. This includes things like spacecraft rotation fixtures, cranes, tilt tables, transport containers, dollies, platforms, and much more. A good example is the Spacecraft Assembly and Rotation Fixture (SCARF) used for the Perseverance Mission. The SCARF is used to lift, rotate, and reposition the hardware for various operational activities. The SCARF is very large so that it can hold hardware and also has high ballymore ladders that are used by personnel to access the hardware. Because of the close proximity and direct contact with the hardware, the SCARF is routinely cleaned and assayed.

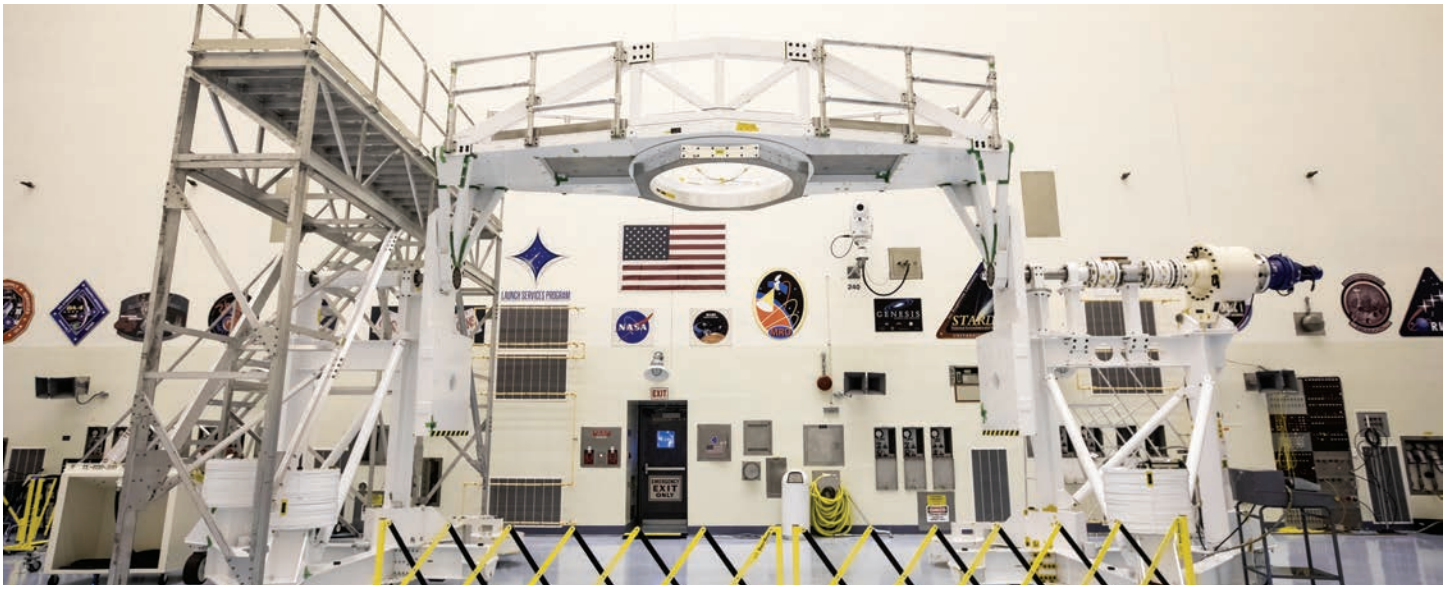

Figure 22: *The Spacecraft Assembly and Rotation Fixture (SCARF) used to process the Mars 2020 rover is photographed inside the Payload Hazardous Servicing Facility at Kennedy Space Center. Attached to the SCARF is an access stand that will allow personnel to reach the spacecraft when it's held above ground level. Photo credit: NASA*

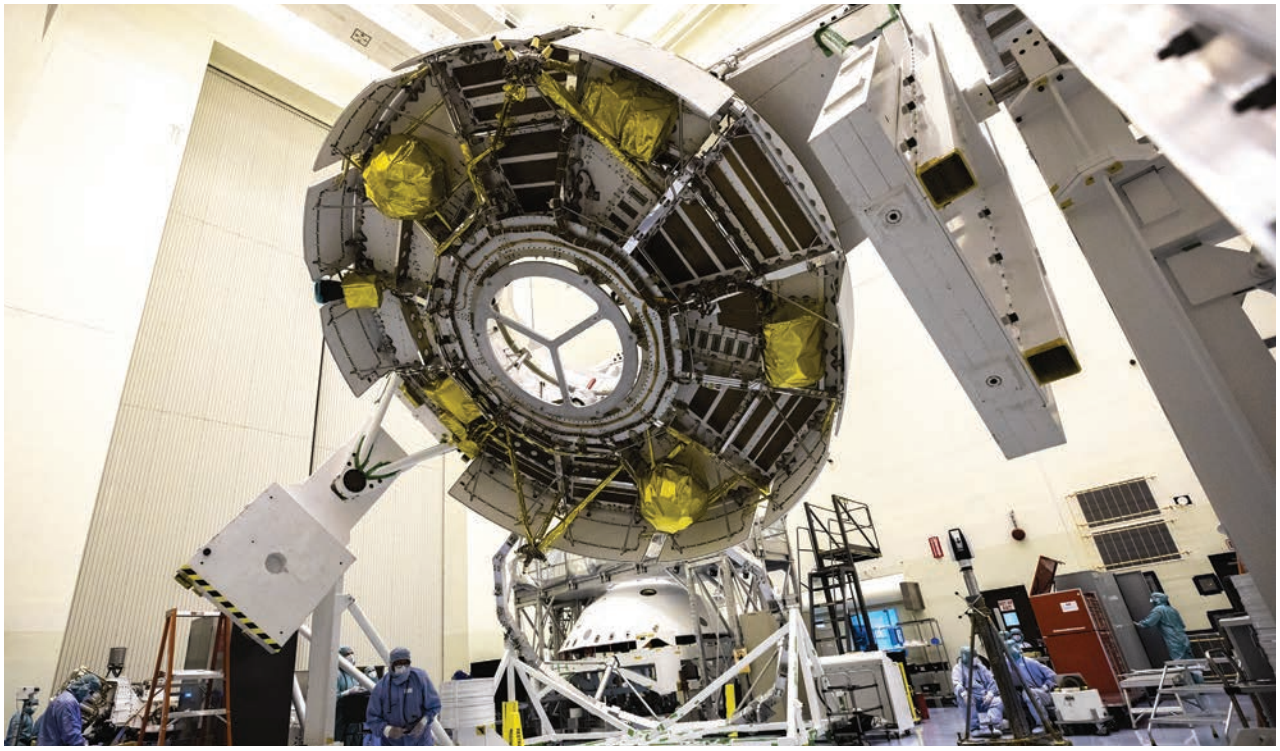

Figure 23: *Functional testing of the Mars Helicopter and its cruise stage occurred in the airlock inside Kennedy Space Center's Payload Hazardous Servicing Facility. The helicopter was tested on a stand while the cruise stage was tested on the SCARF rotation fixture. Photo credit: NASA*

Crane fixtures used to lift and move the hardware also present a cross-contamination concern. With hardware of this size, cross-contamination is controlled by the diligence of the personnel working in the cleanroom, which includes clean gloving, wipe downs of the lift hardware, and covering the hardware during lift operations if needed.

Figure 24: *Technicians using an overhead crane in the Payload Hazardous Servicing Facility at Kennedy Space Center lift the Mars Science Laboratory (MSL) rover, known as Curiosity, for its move to a rotation fixture for testing. Photo credit: NASA*

During assembly, the spacecraft is moved or shipped to different facilities to undergo environmental testing, integrate additional hardware, or prepare for launch. Moving the spacecraft requires a clean moving or shipping container. The container must be fabricated with materials that can be cleaned and wiped down.

Before bringing a container into a cleanroom and placing the spacecraft or hardware into it, the container's internal and external surfaces are cleaned and assayed to verify that it is clean enough to meet cleanroom and mission cleanliness requirements or recleaned as necessary. This process prevents the container surfaces from cross-contaminating the spacecraft surfaces. The spacecraft is also bagged before putting it into a shipping container to add an additional layer of protection.

Figure 25: *Personnel supporting the InSight Mission to Mars load the crated InSight spacecraft into a C-17 cargo aircraft at Buckley Air Force Base for shipment to Vandenberg Air Force Base. Photo credit: NASA*

Figure 26: *Inside the Astrotech processing facility at Vandenberg Air Force Base, the InSight spacecraft was removed from its shipping container and positioned for removal of protective wrapping. Photo credit: NASA*

Other GSE includes hand tools which are routinely wiped and should not be stored or placed on the floor. Tools are stored in clean containers or toolboxes when not in use. Large equipment such as vacuum hoses, electric cables, and other large support equipment is not stored on the floor, and exhaust from items such as power tools, pumps, and blowers is mitigated via the use of high-efficiency particulate air (HEPA) filters or other means of capturing exhaust flows. Avoid tools that shed particulates (i.e., steel wool and bristle brushes).

All day, every day, assembly technicians work in this stringent environment to make sure that flight hardware meets PP requirements. It takes discipline and teamwork to maintain the required environment and to meet assembly and launch schedules.

Figure 27: *Mars 2020 Lift Activities in Payload Hazardous Servicing Facility (PHSF)*
Photo credit: NASA

5.1.3 Hardware Entry

NASA assembles spacecraft hardware such as orbiters, landers, and rovers in cleanrooms. The cleanroom environment controls the amount of particulates, or molecular and biological contamination, to specific requirements.

The cleanroom environment itself and the gowning requirements play a significant role in keeping spacecraft surfaces clean. Building hardware in an ISO-class cleanroom doesn't automatically mean the hardware will remain clean. Other processes must be put in place to ensure the hardware is built and maintained biologically clean.

The cleanroom environment minimizes the biological recontamination potential through air filtration and handling, frequent cleaning of surfaces in the cleanroom, and controlling personnel activities. Many of the activities to contain particles in the cleanroom environment also manage biologicals. Other requirements and activities used during build and test in the cleanroom help meet and maintain strict PP requirements. To understand cleanroom processes, the following definitions are important:

- **Bioreduction:** Removal or destruction of living organisms to some lower population, but not necessarily to zero.
- **Clean assembly:** Practices that minimize contamination from particulates, dust, airborne organisms, or vaporized particles.
- **Bioburden:** The amount of spore-forming bacteria on a spacecraft.
- **Cross-contamination:** Spores or other bacteria unintentionally transferred between objects, such as the transfer from a tool to the spacecraft surface.
- **Assay:** Sampling flight hardware to determine the bioburden on a specific surface area. NASA approves the assay procedures used.
- **GSE:** Ground Support Equipment (such as cables, tools, computers, etc.) that does not fly.
- **Final encapsulation:** The activity associated with closing up the spacecraft to insert it into the launch vehicle (fairings) and staging for launch.

PP requires clean spacecraft hardware assembly practices and prevention of recontamination of the spacecraft up until launch. During assembly, the hardware should be cleaned before entering the cleanroom and maintained clean with regular inspections and status bioassays. Assays to determine the final hardware cleanliness should occur at "last access." Last access describes final installation or the last point in the hardware schedule that a mated surface, subsystem, instrument, or volume is accessible for cleaning and sampling. A mated surface is when spacecraft hardware parts are joined and the surfaces at that junction are no longer accessible for cleaning. PP requires that mated surfaces meet a required and verified level of biological cleanliness. The level is verified by sampling the surfaces with swabs or wipes and assaying for spores before the surfaces are mated.

Assembly teams routinely clean the hardware by wiping with a cleanroom wipe and isopropyl alcohol. Isopropyl alcohol does not destroy bacterial spores in general, but the mechanical action of wiping often removes them. Detergents are not routinely used in the cleanroom due to the risk of residue and because most of them are ineffective against spores unless they specifically contain a sporicide. More importantly, the cleaning solvent must be compatible with spacecraft hardware to prevent any damage. A mixture of 70% Isopropyl Alcohol (IPA) and 30% distilled water is often used for its biocidal and solvent cleaning effect. Hardware is wiped clean before final assembly and installation.

Figure 28: *Wiping down hardware is part of the strategy to limit the number of Earth microbes going to the Red Planet for the Mars 2020 Perseverance Mission. This cleaning takes place in the Spacecraft Assembly Facility cleanroom at the JPL. Photo credit: NASA*

Besides cleaning the hardware surfaces, it's necessary to routinely clean work areas to avoid contamination transfer to hardware. Proper gowning and glove cleaning are required at all times when working with flight hardware.

Maintaining the cleanliness of the spacecraft hardware is a top priority, and all of these activities work toward satisfying PP requirements. Spacecraft hardware can also be protected with clean covers or enclosures when direct access by personnel is not needed. Covering could range from a clean cover over the entire spacecraft, drapes or custom covers for sensitive subsystems, or clean storage bags and bins for parts that have yet to be installed on the spacecraft.

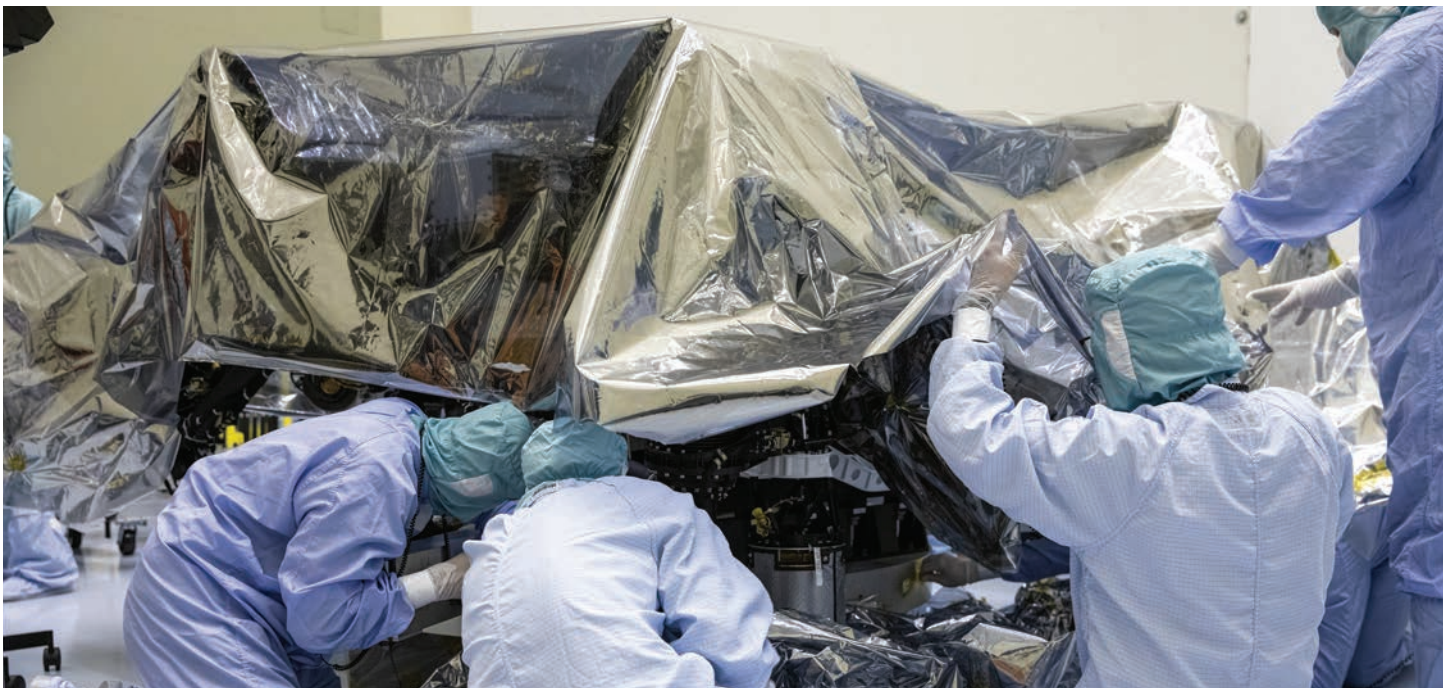

Figure 29: *The Mars 2020 rover undergoes processing inside the Payload Hazardous Servicing Facility at Kennedy Space Center. Note the rover is covered to prevent environmental cross-contamination. Photo credit: NASA*

There are protection measures on the installed spacecraft hardware as well. “Remove before flight” covers serve multiple purposes: they protect the hardware from accidental contact or damage and protect against biological contamination. The Mars Perseverance rover flight wheel treads, which were covered during assembly to prevent biological contamination, are a good example. Covers like this often serve multiple purposes and help hardware to meet both CC and PP cleanliness requirements.

Figure 30: *Mars Perseverance Rover wheel covers (remove before flight). Photo credit: NASA*

HEPA filters isolate closed volumes that by design cannot be cleaned. Usually, the spacecraft hardware vents gases outward or via positive pressure. The HEPA filter protects adjacent hardware from contamination during the mission. The filter captures tiny particles and prevents them from dispersing to other spacecraft areas or to the planetary surface.

Figure 31: *Mars Pathfinder photo on Mars taken by Sojourner camera, showing the integrated subsystem assembly (ISA) HEPA filter. Photo credit: NASA*

Other barriers against recontamination include biobarriers, such as the one deployed on the Mars Phoenix robotic arm. This biobarrier covered the arm to ensure that it remained very clean until deployment. Once Phoenix had landed on Mars and was ready to initiate the arm, the protective biobarrier mechanically opened so the arm could begin operations.

5.2.0 Aseptic Operations in Cleanroom Environments

Aseptic operations are a strategy that can be used to ensure that current bioburden levels on the hardware are maintained during critical integration and test operations.[86,87,88] If the hardware is stored in a biologically controlled but not sterile environment, preparations must be made so that during an aseptic operation, no new biological contamination is introduced. Aseptic operations on flight hardware must undergo the most stringent bioburden verification and use of sterile operation techniques.

Aseptic operations can be instituted as standard practice in a cleanroom or as a discrete event where a workspace is prepared and verified for aseptic work prior to an activity and returned to non-aseptic work after the event.

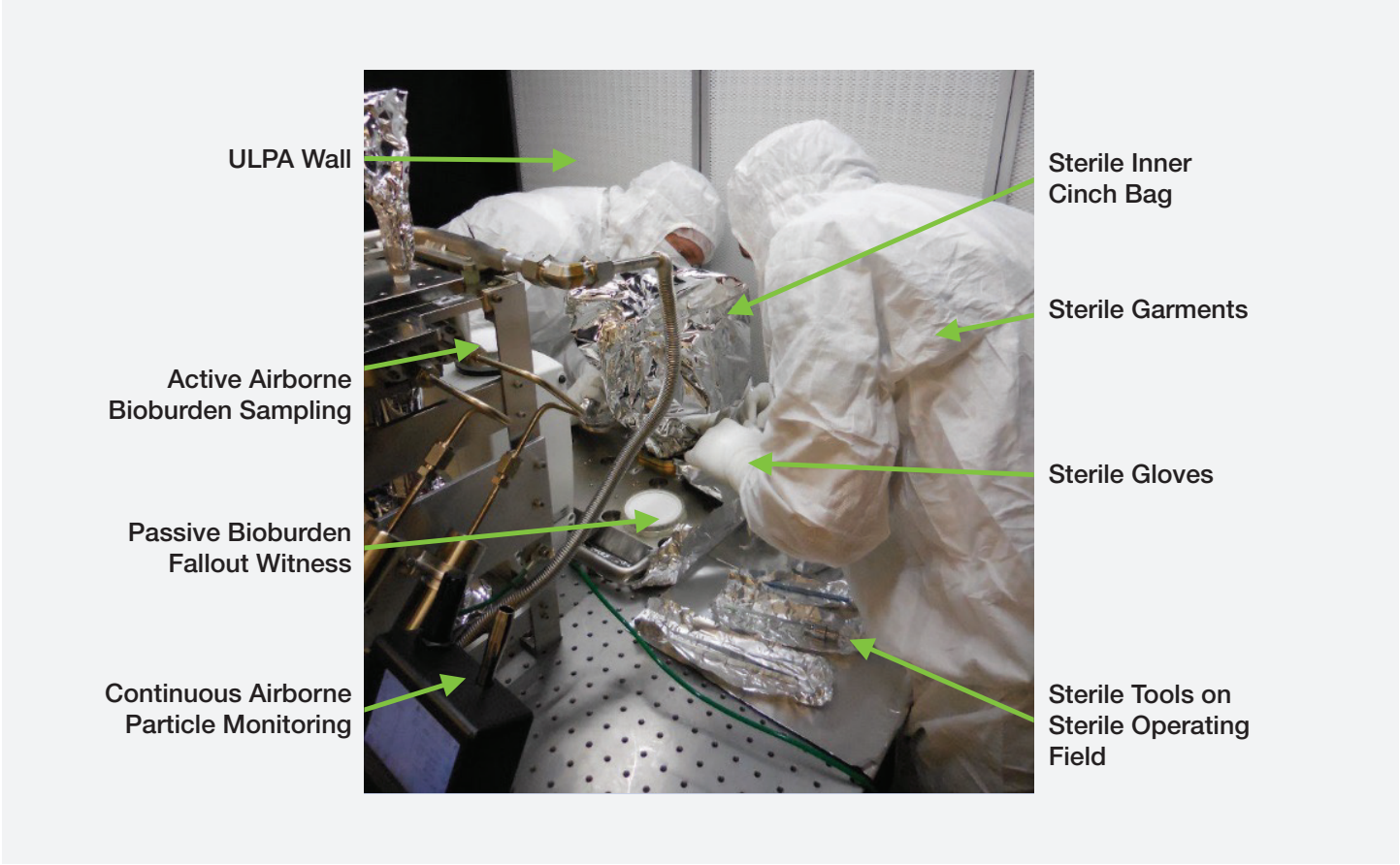

Figure 32: *Aseptic operations during exposure of the sample path of Mars Organic Molecule Analyzer (MOMA) Mass Spectrometer using sterile garments, tools, and monitoring during the operation. Photo credit: NASA GSFC*

Aseptic operations should be implemented to maintain pre-existing sterility or bioburden levels due to (but not limited to) any of the following reasons:

- Mission science or PP requires sterility of the hardware being accessed.
- Hardware has already undergone final DHMR or other bioburden reduction process, resulting in a bioburden level below the limits of detection for a standard swab assay.
- The bioburden was verified to meet requirements and surfaces cannot be recleaned or resampled.

[86]Lalime, Erin N, John S Canham, and Radford L Perry. 2018. "Development and Implementation of Aseptic Operations to Meet Planetary Protection Requirements on the MOMA-Mass Spectrometer (Conference Presentation)," September.
[87]Lalime, Erin. 2016. "Establishing and Monitoring an Aseptic Workspace for Building the MOMA Mass Spectrometer." Proceedings of SPIE, September.
[88]Chen, Fei, Cynthia Ly, Ioannis Mikellides, Douglas Bernard, and Moogega Cooper. 2023. "Mars 2020 Mission Biological Return Sample Contamination Control Approach and Verification." Astrobiology 23 (8): 862–79. https://doi.org/10.1089/ast.2022.0048.

Guidelines for establishing an aseptic work environment can be found in ISO 13408,[89] FDA regulations 21CFR211.42,[90] as well as in "Annex 6 WHO good manufacturing practices for sterile pharmaceutical products."[91] The work environment should have surfaces that are cleanable and the air should be laminar flow HEPA or ultra-low particulate air (ULPA) filtered to ISO Class 5 air quality.[92] Molecular air quality may also be required depending on the sensitivity of the hardware or instrument.

Aseptic operation approach and validation levels should be described in a mission PP implementation plan. Guidelines for setting action levels for aseptic operations can be found in ECSS-Q-ST-70-58C Bioburden Control of Cleanrooms.[93] ECSS-Q-ST-70-58C recommends that aseptic operations occur in an environment that is verified to have < 1 CFU/m^3 for air samples and < 400 CFU/m^2 for surfaces.

In preparation for aseptic operations, any unnecessary equipment or clutter should be removed and non-sterile surfaces isolated away from the operation using a biobarrier such as sterile drapes or sterile bags. All surfaces and exposed equipment should be cleaned with biocides, and both air and surface bioburden should be verified to meet predefined validation levels.

All tools used during an aseptic operation should be prepared for sterile handling. Most tools should be sterilized while double-wrapped and not opened until needed during the aseptic operation. There are multiple acceptable approaches to sterilizing tools, including heat sterilization (DHMR or autoclave) or VHP as long as it meets industry standards. Some tools or equipment may not be compatible with standard sterilization techniques, so these surfaces should be isolated behind biobarriers such as sterile foil or bagging. Surface isolation can be used for handles or similar structures, but not the parts of the tool directly interfacing with the sensitive hardware. Any surface directly in contact with sensitive hardware should be compatible with sterilization.

Entry to the aseptic workspace should be limited to only critical operators during aseptic operations. Sterile cleanroom garments (disposable or reusable) should be used for a single aseptic operation before disposal or resterilization. Gloves should be sterile and double gloved so that the outer glove can be changed as often as necessary. Sterile goggles are also recommended. Once sterile garments and gloves are donned, care should be taken to not touch any non-sterile surface or change gloves if non-sterile surfaces need to be touched.

During aseptic operations, the immediate environment should be monitored. A real-time particle counter can be used to ensure that ISO Class 5 particle levels are maintained, and airborne bioburden should be monitored using an active and passive air sample strategically placed to capture the environment relevant to the most sensitive surfaces. These monitors should be exposed for the duration of the aseptic activity. Sheets of sterile foil (or other sterile materials) could be utilized to provide sterile surfaces for hardware assembly to be conducted on, as well as sterile covering and packaging.

Accessing and handling of sterilized tools needs to be done with care. The exterior of the sterilization container (foil, autoclave bag, etc.) will not be sterile, so it is recommended that a second operator open the outer layer and the primary sterile operator handles only the sterile inner layer and the tool itself. After being opened, the tools should touch only verified hardware or other sterile surfaces.

If an aseptic operation is time-sensitive, the amount of time that the critical surface is exposed should be documented.

At the conclusion of an aseptic operation, once all sensitive surfaces are closed, post-aseptic operation monitoring should be conducted to ensure that sterile conditions were maintained. Air samples taken during the operation and surface samples of the work environment taken at the completion of aseptic operations can be used to verify that the environment remained free of biological contamination during the operation. Contact plates should be collected from the glove prints of the primary sterile operator who has direct handling of the hardware. Active and passive air samples and contact plates should be transported to the PP lab, prepared with sterile TSA, and incubated at 32 °C for 72 hours. If any CFUs are detected on the plates, it indicates that a contamination incident happened during the associated assembly activity. Particle count data should also be reviewed to ensure that ISO Class 5 was maintained.

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[89]14:00-17:00. n.d. "ISO 13408-1:2008." ISO. **https://www.iso.org/standard/37842.html**

[90]"CFR - Code of Federal Regulations Title 21." **www.accessdata.fda.gov**. Accessed October 21, 2021. **https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=211.42**.

[91]"Annex 6 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products." **https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/production/trs961-annex6-gmp-sterile-pharmaceutical-products.pdf**.

[92]International Organisation for Standardisation, "ISO 14644-1 Classification of air cleanliness by particle concentration," *International Standard: Cleanrooms and associated controlled environments*, 2015.

[93]"ECSS-Q-ST-70-58C – Bioburden Control of Cleanrooms (15 November 2008) | European Cooperation for Space Standardization." Ecss.nl. Accessed February 6, 2024. **https://ecss.nl/standard/ecss-q-st-70-58c-bioburden-control-of-cleanrooms/.**

For the most stringent aseptic operations, hardware may be processed inside of a sterile glovebox (Figure 33 below). For glovebox operations, all hardware and tools should be sterile before entering the glovebox. This is often achieved with a two-sided sterilization chamber, one side of which is open to the ambient environment and the other which opens into the glovebox.

Figure 33: *Honeybee Robotics glovebox used during Mars 2020. Photo credit: Honeybee Robotics*

5.3.0 Macroorganisms

NASA Standards require that foreign object debris (FOD), including operational debris, environmental debris, and macroorganisms (e.g., birds, reptiles, bats, rodents, insects, spiders, and mites) be addressed in PP pre-launch reporting requirements. Assembly facilities may meet designated particulate control requirements as outlined by ISO standards, but finding instances of macroorganisms in these controlled environments is unacceptable for PP purposes. Birds, small reptiles, insects, and rodents can and have found their way into certified cleanrooms, test facilities, and flow benches. These events are significant operational anomalies potentially affecting PP compliance. Such anomalies are documented, particularly to evaluate the risk to the spacecraft and its associated hardware in the area where the anomalies occurred. Corrective action and mitigation plans are documented and implemented with the goal of revalidating hardware compliance and preventing additional macroorganism occurrences.

Ingress opportunities into these areas vary, but transfer of hardware between airlocks that open from the outdoors into cleanroom high bays or other controlled assembly environments provides one possible entry route. Airlock operations require vigilance when doors are open to the outside or when hardware, GSE, or supply containers are moved to and from the area. Environments at the facilities vary; for example, the humid environment at Cape Canaveral is rich with bug infestations, including mosquitos, *Plecia nearctica* Hardy (known as “love bugs”), and other flying insects. In addition to ingress through open doors, protective covers on hardware during moves can travel through swarms of bugs during transport. The cover creates a similar effect to the impact of a bug on the windshield of a car and can be covered with bugs even during

short-duration transport activities. Operationally, the covers are removed or cleaned prior to ingress into the airlock. Transport containers and covers benefit from construction using materials that are easily wiped down and cleaned. Wood shipping containers, even if painted, are discouraged as they are very difficult to clean and may serve as a habitat for macro or microorganisms. Hardware inside the airlock is verified free from macroorganisms prior to moving into the clean facility.

Certified cleanrooms, benches, and other controlled assembly areas benefit from the project inspecting and addressing macroorganism potential prior to hardware assembly. In addition to doors and other entrances, cleanrooms should be initially and routinely inspected for other ingress opportunities. Potential entry areas include open grates, wall penetrations, and perforated covers. Wall mounts that penetrate surfaces, or areas around the penetrations that are not sealed represent potential ingress or habitation locations. Even very small cracks in walls or around door seals can allow bugs, spiders, and other unwanted insects into the area. If areas have openings or gaps, they should be repaired or temporarily taped, caulked, or covered to prohibit unwanted ingress. Routine inspection and wiping of equipment and tools that move into the cleanroom from the change area are also beneficial in preventing stowaway macroorganisms and the control of bioburden in general. General housekeeping is also important in mitigating macroorganism issues. Often, cleanrooms have areas where hardware, GSE, and other operational equipment are staged or stored. If the storage areas are crowded or impede routine cleaning activities such as mopping and wipe downs, the negated areas may provide undetected habitats for spiders, beetles, or other small insects. Unexpected weather or environmental events can also trigger an influx of macroorganisms. This can lead to transport via leaks or flooding or creatures that are stressed and looking for shelter. Because macroorganisms present an extreme risk of contamination to flight hardware, PP requires the project take a proactive approach to mitigate any ongoing macroorganisms' ingress or presence in the cleanroom. If macroorganisms are observed, the project must document the event with a pre-developed protocol that minimally includes:

1. Type and location of macroorganism.
2. Removal and specimen analysis if safe to do so.
3. Inventory of the affected hardware.
4. Risk assessment of hardware based upon macroorganism observations.
5. Mitigation plan to prevent additional ingress.
6. Mitigation plan for hardware, including required PP assays, hardware cleaning or reprocessing, covering, and/or removal until the situation is corrected.
7. Inform key stakeholders (project management, CC, SMA, OPP, etc.) of the event and outcome.

Industry models of inspection and mitigation practices provide a process example and a strong framework for risk abatement in PP.[94] A general industry approach is provided below.

General Methods of Macroorganism Contamination Control

Risk assessments should be considered when addressing macroorganism biological contamination control in cleanrooms and associated spacecraft environments. The hazard analysis critical control point (HACCP) system is commonly used in industry for biological control.[95,96,97] Fault tree analysis (FTA) or the failure mode and effect analysis (FMEA) are widely used alternatives to the HACCP. Each mission may have a tailored risk assessment establishing a baseline contamination level, monitoring plan, and risk zones. The objective for the risk assessment is to enable missions in the planning stages to focus on prevention and proactive measures to minimize biological contamination while establishing alert and action levels with a clear reporting and mitigation strategy for rapid responses.

Pest control is the main form of biological contamination control in industry, with a focus on integrated pest management (IPM), which includes strategic approaches to continually minimize any form of contamination. The IPM should not only identify the detailed pest information, but also address the associated mitigations to reduce the pest threat in the future. In addressing pest management strategies, the following should be included as part of the plan: exclusion, sanitation,

[94]International Standard: Cleanrooms and Associated Controlled Environments, Biocontamination Control. Accessed February 18, 2024. **https://www.iso.org/standard/25015.html**.
[95]Pierson, M, and D Corlett, eds. 1992. HACCP — Principles and Applications. Van Nostrand Reinhold, New York.
[96]1995 Codex Alimentarius Commission. "Hazard Analysis Critical Control Point (HACCP) system and guidelines for its application." Alinorm 97/13. Annex to Appendix II. Joint FAO/WHO Food Standards Programme. Rome, Food and Agricultural Organization of the United Nations, 1995
[97]Jahnke, M. "Use of the HACCP concept for the risk analysis of pharmaceutical manufacturing process." European Journal of Parenteral Sciences, 2(4), pp. 113-117, 1997

landscaping, and the formulation of an ongoing inspection schedule.[98,99] Exclusion includes sealing gaps and installing double doors and air curtains. Sanitation covers the removal of dirt, webs, food, spills, garbage, and standing water that offers both sustenance and breeding opportunities. Planned landscaping allows a facility to create a natural – and often aesthetically pleasing – barrier to unwanted guests that discourages organisms in this zone. This includes not only the obvious ones such as mice and rats, but also termites, which need to construct an earth tunnel to move from place to place. And finally, the ongoing inspections can serve to address pest infiltration in a timely manner, thereby minimizing the risk of a large-scale infestation.

In addition to an active pest management plan, additional measures can be deployed to help reduce/suppress intrusions into and within the cleanroom environment. External measures may include modifications to the exterior landscaping, improvements in sanitation, identification and sealing of cracks and expansion joints, improving the sealing around the cleanroom, evaluating exterior lighting to avoid attracting insects to the building, and evaluating the facility's air-handling and air-filtration system. A detailed landscape plan should also be evaluated, as mowing or pollinating plants can cause organics to be ingested into the cleanroom's air-handling system. Interior measures may provide an additional preventative layer against macroorganisms to include UV lights for insects, radar systems for mice, bait inside detection boxes to track location and species of invading macroorganisms, glue traps with or without species-specific pheromone bait, and heat treatment. For crawling insects, bait and glue traps and insect detector boxes are typically deployed. For birds, a species-specific approach is needed and may include electric wire and nest-deterrent systems (like stainless steel spikes).[100] Pesticides are usually considered a last resort not only because they could pose an occupational hazard, but also because they could further contaminate the spacecraft with unwanted inorganic and organic residues.

After an exposure occurs, the collection and evaluation of the specimen, reporting, and mitigations should be considered. Collection of the specimen will help identify the pest, and the report should list what is known about that pest's biology, behavior, food choices, and breeding conditions. The identity of the pest found in a cleanroom may also point to a biological or behavioral feature of the pest that will indicate its likely source and suggest remedial measures. Reporting should include a detailed assessment of the insect, spacecraft or cleanroom impacts, remediation measures, and any updates in monitoring or mitigation planning.

6.0.0 NASA Standard Assay Laboratory Considerations

Navigation Summary: *Chapter 6 provides details on implementing the NSA. This chapter is applicable to PP microbiology laboratory practitioners supporting Category III, IV, or IV missions.*

Use of Bacterial Endospores

Bacterial Endospores: *Robust and metabolically dormant bacterial forms produced from actively replicating bacteria as a response to environmental stressors. Heat-resistant spores are detected using the NASA Standard Assay as an indicator of spacecraft biological cleanliness.* (NASA-STD-8719.27 definition)

Bacteria represent a significant portion of life on Earth. While they are invisible to the naked eye, they are prevalent in the environment and can have both positive and detrimental effects on humans. It is estimated that a gram of soil may contain approximately 40 million bacterial cells.[101] Similarly, a milliliter of fresh water may contain a million bacterial cells.[102] Even humans harbor large quantities of bacteria, both on the skin and internally (of the order 1 x 10^{13}/person).[103]

There are many types (classifications) of bacteria, all of which are capable of replication and cell division over time. Bacteria may be categorized by their morphology or by other characteristics like metabolism or environment, or increasingly by their DNA sequence.

[98]Health Facilities Mgmt. "Pest prevention tips for health care facilities." ASHE, Environmental Services. April 5th, 2017.
[99]Clark, Michael. "Bioremediation of Industrial Pollutants by Insects Expressing a Fungal Laccase." ACS Synthetic Biology. ACS Biol 11, 308-316. 2022.
[100]Schuerger, Andrew. "Integrated Pest Management Protocols for Space-Based Bioregenerative Life Support Systems." *Sec. Astrobiology* Volume 8 - 2021.
[101]Raynaud, Xavier, and Naoise Nunan. 2014. "Spatial Ecology of Bacteria at the Microscale in Soil." Edited by Francesco Pappalardo. PLoS ONE 9 (1): e87217. **https://doi.org/10.1371/journal.pone.0087217**.
[102]Rodriguez-Valera, Francisco. 2011. "Bacteria." Encyclopedia of Astrobiology, 137–39. **https://doi.org/10.1007/978-3-642-11274-4_143**.
[103]Sender, Ron, Shai Fuchs, and Ron Milo. 2016. "Revised Estimates for the Number of Human and Bacteria Cells in the Body." PLOS Biology 14 (8): e1002533. **https://doi.org/10.1371/journal.pbio.1002533**.

Bacterial endospores (referred to as spores throughout) are a subcellular body that some species of bacteria form to defend against conditions unfavorable to their lifecycle. Spores are formed when a bacterial cell encounters environmental challenges that do not favor growth or cell division.

Spores are resistant to many environmental challenges, including temperature extremes, drying, radiation, and even chemical exposures such as acids and chemical disinfectants. Instead of dividing and replicating, a vegetative (active) cell sporulates when the required growth environment is detrimental to these activities. Once sporulation occurs, the spore can remain dormant for possibly tens of thousands of years or longer.[104]

Spores can and will remain dormant until conditions for growth once again present themselves; they can convert back to active vegetative cells very quickly when conditions become favorable. Even when exposed to bioburden reduction treatments, spores can repair DNA and other cellular damage upon germination.[105,106] Because of this natural defense mechanism, spores are likely candidates to survive harsh conditions, including dry, high-temperature, or radiation environments and planetary launch and landing scenarios such as those found on planetary missions. We know that spore-forming bacteria are part of the human biome, can be carried on skin or clothing, or may be transferred via unclean GSE, flight hardware, and air circulation systems.

Because of spore-forming bacteria's potential survivability during launch and in space environments, together with their ease of use in a microbiological laboratory, NASA's analytical requirements for bioburden use spores as an indicator of spacecraft biological cleanliness.

The NSA is used to isolate, culture, and account for spore-forming bacteria collected from flight hardware, ground support systems, and other relevant areas associated with flight hardware assembly, test, and launch operations (ATLO).

Laboratory

The NSA requires access to a laboratory equipped to do culture-based microbial analysis. Ideally, the laboratory is in close proximity to the primary sampling location. The laboratory has designated work, storage, and office areas with the appropriate quality controls to ensure cleanliness. The laboratory has the equipment needed to conduct assays and adequate work and storage areas to support required assay throughput using aseptic techniques. General laboratory QA/QC practices should be established and implemented in a Laboratory QA Plan.

Laboratory Equipment

A primary equipment list utilized in the NSA is provided in Table 8. Operations involving the manipulation of sterile items and sample processing are performed in laminar flow hoods or environments meeting Class 100 air cleanliness requirements. Personal protective equipment (PPE) includes laboratory coats and smocks, gloves, hair bonnets or hoods, and face covers and is donned in accordance with the laboratory's documented procedural requirements. General laboratory supplies include standard laboratory glassware, test tubes, Petri plates, media bottles, pipettors, and cleaning supplies. Data management supplies include everything from general office supplies to computers. An example laboratory general supply list is included in Table 9.

[104]Cano, R J, and M K Borucki. 1995. "Revival and Identification of Bacterial Spores in 25- to 40-Million-Year-Old Dominican Amber." *Science (New York, N.Y.)* 268 (5213): 1060–64.
[105]Setlow, B, and P Setlow. 1996. "Role of DNA Repair in Bacillus Subtilis Spore Resistance." Journal of Bacteriology 178 (12): 3486–95. **https://doi.org/10.1128/jb.178.12.3486-3495.1996**.
[106]Nicholson, Wayne L, Andrew C Schuerger, and Peter Setlow. 2005. "The Solar UV Environment and Bacterial Spore UV Resistance: Considerations for Earth-To-Mars Transport by Natural Processes and Human Spaceflight." Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis 571 (1-2): 249–64. **https://doi.org/10.1016/j.mrfmmm.2004.10.012**.

Table 8: *Essential Laboratory Equipment for NSA*

Autoclave	Laminar flow cabinet(s)	Vortex
Colony counter	Refrigerator	Water bath(s)
Laboratory dishwasher	Sonicator	Weighing balance (0.1-400g)
Ice source	Stereomicroscope	Mediaclave/media sterilizer
Incubator	Storage cabinets	

Table 9: *General Laboratory Supplies Used To Conduct the NSA*

Autoclave biological indicator (glassware)	Gloves (extra-large)	Plate counting pens (black)
Autoclave biological indicator (media)	Graduated cylinders (500 mL)	Plate counting pens ink refill (black)
Autoclave sterilization bags	Graduated cylinders (1000 mL)	Plate counting pens ink refill (red)
Autoclave sterilization indicators	Hand truck	Polyoxyethylene sorbitan monooleate
Autoclave tape	Inoculating loops (2 id)	Potassium dihydrogen phosphate
Bacterial standards	Inoculating loops (5 id)	Rulers (measuring)
Beakers, Griffin (1000 mL)	IPA bulk	Scissors
Beakers, Griffin (2000 mL)	IPA-saturated cleanroom wipe	Step stools
Beakers, Griffin (600 mL)	Lab coats	Sterile spoons/scoops, disposable
Bench paper (30-inch)	Lab scoop	Sterilization basket (105 x 123 x 154 mm)
Biological indicator incubator	Media bottles (1000 mL)	Sterilization tray lids
Bunsen burners (natural gas)	Media bottles (500 mL)	Sterilization trays
Bunsen lighters (strikers)	NaOH, sodium hydroxide (1N)	Stools, lab sitting
Bunsen tubing (per gas requirement)	Parafilm (2 x 250)	Tacky mats (18” x 36”)
Cotton-tipped applicators (sampling)	Parafilm (4 x 250)	Test tube racks
Dishwasher soap	Pen, lab mark (x fine black)	Test tubes
Disposable pipettes (sterile 2 mL)	Petri dishes (100 x 15 mm)	Thermometers (-20 °C/105 °C)
Disposable pipettes (sterile 10 mL)	Petri dish racks	Thermometers (20 °C/110 °C total immersion)
Disposable pipettes (sterile 25 mL)	Pinch cutters	Trash cans (white)
Disposable pipettes (sterile 5 mL)	Pipet-Aid replacement filters	Trypticase Soy Agar
Flasks, Erlenmeyer (125 mL)	Pipette tips (101-1000 sterile)	Wash bottles, poly (500 mL blue)
Flasks, Erlenmeyer (250 mL)	Pipette tips (1-100 uL sterile	Wash bottles, poly (500 mL green)
Flasks, Erlenmeyer (50 mL)	Pipettor carousel	Waste bags, floor (25/35), autoclavable
Forceps (fine point)	Pipettors, digital-pipet aid	Waste bags, bench top (8 x 10)
Glassware cart (4-basket)	Pipettors (.1-2.5 uL)	Water bath timers
Gloves (large)	Pipettors (.5-10 uL)	Weighing boats
Gloves (medium)	Pipettors (100-1000 uL)	
Gloves (small)	Pipettors (10-100 uL)	

Aseptic Technique in Processing NSA Samples

Aseptic techniques *– A collection of design practices and laboratory protocols and procedures intended to prevent the introduction of bacteria and other contaminants during a specified process, thus keeping the environment (and articles in the environment) sterile.*[107]

Bacteria are everywhere, with humans being a significant source of possible biological contamination during sampling and in the laboratory. PP uses standard aseptic techniques during sampling and in the laboratory to prevent and reduce the probability of contamination during sample processing and culturing. Aseptic approaches include environmental controls such as air filtration and control in laminar flow hoods, flaming equipment (using a Bunsen burner), and environmental barriers such as gloves, laboratory coats or gowns, and face masks.

PP uses filtered and controlled airflow hoods. Laminar flow hoods are most often used and provide directed HEPA-filtered air to minimize cross-contamination and to aid in maintaining a sterile and contaminant-free environment. Key components of laminar flow hoods include:

- HEPA filter which limits air particulates of sizes greater than 0.3 micrometers (μm) in diameter
- Fan to move air through the filtration system
- Screen that produces a laminar airflow
- Ultraviolet light and florescent light: UV to aid in sterilization of interior surfaces when not in use and a fluorescent work light

Barriers

Physical barriers prevent the transfer of microbial contamination from the environment and from the analyst during laboratory activities. PP requires physical barriers, including sterile gloves and appropriate PPE during sample processing, sterile sample tools, pipettes, glassware, Petri plates, and any item with the potential to contaminate the sample during processing. Sterile barriers and equipment are cleaned and packaged to maintain sterility and have not touched a contaminated surface prior to use. The barriers or laboratory equipment are discarded or re-sterilized after use. Laboratory DQOs and the laboratory quality implementation plan will address requirements specific to aseptic techniques.

Method Implementation and Process Validation

Qualifying the Method in the Laboratory

PP analytical methods are developed and systematically implemented using industry standard performance characteristics. This process confirms the NSA generates data that is acceptable for its intended use. The method is validated in the laboratory and verified periodically over the use lifetime to ensure that the method is operating properly.

Reagent and Laboratory Preparation

During analysis procedures, significant contamination can result from poor sample handling. Preparation for sample work-up uses aseptic principles to prepare glassware and reagents needed to perform the NSA. Supplies required for the assay include media bottles or appropriate tubing used for a mediaclave, Petri plates, test tubes, and other miscellaneous laboratory supplies. Items are either sterilized by autoclaving or purchased and maintained sterile. Liquids including agar (growth media), sterile rinse solution, and deionized (DI) water are also sterilized prior to use. In practice, the performance of each autoclave should be validated and routinely monitored with indicator organisms under various conditions of loading to establish documented operational requirements for the method. In most cases, the manufacturer's instructions provide adequate detail on the validation and temperature requirements for sterilizing materials and liquids.

Water Quality

PP requires that water laboratory systems meet ASTM type II water purity as outlined in **ASTM D1193-06**. Commercial laboratory filtration systems provide purified water at required ASTM resistivity, conductivity, and total organic carbon levels.

[107]ISO-14644-1:2015. Cleanrooms and associated controlled environments - Part 1: Classification of air cleanliness by particle concentration.

The laboratory water purification system should be operationally validated and verified using independent sampling techniques prior to use on flight projects. This validation confirms that the system consistently and reliably produces the desired water quality. Once in use, routine system verification and monitoring is implemented using operational control limits and routine independent verification sampling to ensure that it is operating in accordance with the laboratory and flight project's water quality requirements.

Sterile Rinse Solution

Sterile rinse solution is a buffered sterile water that is used with 200 mL aliquots in the wipe extraction process during sample processing. The solution is prepared in the laboratory in two steps:

1. Preparation of a phosphate-based buffer solution
2. Preparation of sterile rinse solution, which incorporates the phosphate buffer, ASTM II grade water, and polyoxyethylene sorbitan monooleate surfactant

Both solutions are prepared in accordance with NASA-STD-8719.27. The buffer solution is not sterilized once it is made. The rinse solution is sterilized in accordance with the laboratory's liquid sterilization requirements prior to use.

Agar

Tryptic soy agar (TSA) is the medium used to promote bacterial growth. It is a powder-based material containing nutrients and agar that is mixed with water and autoclaved. As the media cools, it forms a gelatin-like consistency. There are two ways that agar is used in PP assays. The NSA plates the extracted sample by inoculating (the process of introducing microbes into a culture media) the sample solution with molten agar, which subsequently solidifies. An alternate approach of membrane filtration has also been approved for use in processing NASA cleanroom samples, which involves capturing microorganisms from a sample on a membrane and placing that membrane onto solidified agar.[108]

NSA

The NSA uses standard microbial techniques in the analysis, including heat shocking and plating of samples, to grow and enumerate the spore-forming bacteria. The lab work has several components, including reagent and laboratory preparation, spore isolation, culturing, and enumeration/data analysis.

The NSA protocol is found in NASA-STD-8719.27.

Extraction

Microorganisms on a surface are collected by abrasively removing them from the spacecraft surface onto the wipe or swab. The first step of the assay process is to remove the microorganisms from the surface of the sample tool. Swab samples are extracted using the 10 mL of sterile deionized water used during sampling. Wipe samples are extracted in sterile rinse solution (a buffered water), which is added in the laboratory. Extracting bacteria from the surface of the sample tool begins with approximately five seconds of vortexing. The sample container is placed on the vortexer, which provides energy that creates a circular movement (a vortex) around the swab to start particle or microorganism removal.

The second step in the extraction process is sonication. Samples are suspended in an ultrasonic bath, making sure the liquid level in the bath is above the fluid level in the sample container and that the number of samples does not exceed the performance rating of the sonicator. Sonicate for 2 minutes +/- 5 seconds.

The sonicator and all assay equipment is operated and maintained according to the manufacturer's instruction and the laboratory's QA plan. In general, the sonicator fluid contains water and aqueous solution of 0.02 percent volume-to-volume polysorbate 80 and is changed daily. The sonicator is run on the degas cycle for five minutes prior to the start of use for the activity (before loading samples) or after changing the bath solution. The sonicator is operated within a temperature range of 20-32°C. The sample tubes or bottles are suspended in the water (not placed on the floor of the sonicator) with adequate space so that they do not touch. The samples are sonicated for 2 minutes +/- 5 seconds.

[108]Stott, Kristina Vaikovna, Lyssa Morgan, Caitlin Shearer, Morgan Byrd Steadham, Mihaela Ballarotto, and Ryan Hendrickson. 2022. "Qualification of Membrane Filtration for Planetary Protection Flight Implementation." *Frontiers in Microbiology* 13 (April). **https://doi.org/10.3389/fmicb.2022.871110**.

Past PP handbooks have specified that the ultrasonic water bath use 25hz frequency as well as other specifications on the size and composition of the water bath,[109,110] which may not match currently available commercial units. Ultrasonic extraction is a key component to sampling and processing efficiency, and laboratories should be able to demonstrate sample efficiency with their specific equipment configuration. Tests and modeling to establish efficiency are discussed in Dinacola et al[111] and use 25hz frequency for ultrasonic extraction. Use of other frequencies may require additional efficiency testing.

Spore Isolation

The next step in the assay is to separate bacterial endospores (spores) from other types of microorganisms. This is accomplished using a heat shock step. Spores are able to tolerate higher temperatures than many types of bacteria. Exposing the extraction liquid to heat for a specific amount of time effectively kills most vegetative (non-endospore) organisms. The spores survive and can then be cultured in the laboratory.

The heat shock process requires that samples be heated to 80 °C ± 2 °C for 15 minutes as monitored by a pilot tube or bottle. A heated water bath is used to submerge the sample container up to the liquid line in the container. A pilot reference uses a container identical to those used for the assay samples, filled with the same volume of extraction solution used for the test samples. A thermometer is be placed in the pilot reference, which is used to monitor the temperature of the samples during the heat shock process. Samples are heat shocked and then removed and placed in an ice bath to quickly quench the heating process. After heat shock, the samples are cooled rapidly on ice to bring the contents to 30-35 °C. This effectively stops the die-off of bacteria from heat. The spores are effectively isolated and can be plated and enumerated.

Plating

The next step in the assay process is plating the extraction liquid. There are several key concepts. The first is pour fraction. The pour fraction reflects the amount of sample plated in relation to the total sample. The total sample is measured as the sample volume of the extraction liquid, which is 10 mL for swabs and 200 mL for wipes. The pour fraction is the amount of liquid plated in relation to the total volume. The minimum required pour fraction is 0.8, which requires that 8 of the 10 mLs of extraction fluid be plated. The minimum pour fraction for wipes is 0.25, which requires 50 of 200 mLs be plated. Petri plates come in a number of sizes; 100 mm is commonly used for the NSA and multiple plates are poured for each sample. In general, the loading for a plate is no more than 4 mL of sample to approximately 20 mL of liquid agar. This ratio allows the agar to solidify. Sample volumes greater than 4 mL/plate may dilute the agar sufficiently so that it will not solidify appropriately. Usually 2-4 mL of sample solution is plated, which means that 2-4 plates are used for swab samples (8 mL in total) and 13-25 plates for wipe samples (50 mL in total). Petri plates are inoculated with the sample so that the spores can begin to grow and be counted as colony-forming units (CFU).

Bacteria require specific growth conditions, which include appropriate nutrients and temperature. There are many types of nutrient agars used to culture bacteria. The NSA requires that TSA be used. The agar powder is weighed and mixed with water per the manufacturer's instructions. The liquid agar is autoclaved per the manufacturer's instructions and maintained at 40-50 °C so that it remains fluid. All plating activities are conducted in a laminar flow hood with PPE such as gloves and lab coats to prevent contamination. Extreme care should be taken during this process to maintain equipment as presterilized to minimize the risk of sample or Petri plate contamination. The plating process is as follows:

1. Label the Petri plate with the sample identifier.
2. Using a sterile pipette, aliquot the appropriate amount of sample into the Petri plate.
3. Add approximately 20 mL of sterile, molten (48-50 °C) TSA to each plate; mix the contents by gently swirling and allow the mixture to solidify at room temperature. The samples should be covered with the Petri plate lid whenever possible to minimize contamination risk.

[109] **https://explorers.larc.nasa.gov/2019APSMEX/SMEX/pdf_files/NASA-HDBK-6022b.pdf**

[110] Puleo, J R, N D Fields, Sune Bergström, Gordon S Oxborrow, P. Stabekis, and Robert Koukol. 1977. "Microbiological Profiles of the Viking Spacecraft." *Applied and Environmental Microbiology* 33 (2): 379–84. **https://doi.org/10.1128/aem.33.2.379-384.1977**.

[111] DiNicola, Michael, Arman Seuylemezian, Lisa Guan, Christine Moissl-Eichinger, Amy Baker, and Jason Johns. 2023. "Modeling of Recovery Efficiency of Sampling Devices Used in Planetary Protection Bioburden Estimation." *Applied and Environmental Microbiology* 89 (12). **https://doi.org/10.1128/aem.00832-23**.

Controls

The NSA requires sterility, positive and negative control preparation, and processing for each assay conducted. The controls provide information that samples have not been contaminated during collection or processing. Sterility controls include a field blank and laboratory blank. The positive control confirms sample processing and associated media will support bacterial spore growth. The negative control demonstrates that media is clean from bacterial contamination during processing.

- **Laboratory blank:** Determines if bacterial contaminants are present in the laboratory environment, the reagents, or the apparatus used during processing. An unused and transported swab or wipe is prepared in the laboratory as a surrogate sample and carried through the analysis with the samples.
- **Field blank:** Determines if preparation, transport, or environmental contamination is present during the sample collection process. The field blank is an unused sample wipe or swab handled, transported, and analyzed as if it were a sample.
- **Positive Control:** Prepared using spore standards such as *Bacillus atrophaeus* ATCC 9372 (DSM 675). A sample containing a known quantity of the spore standard is prepared and carried through the laboratory procedure. The CFU recovery indicates that the media can support the growth of the organism of interest. The CFU recovery is captured and evaluated against laboratory method quality requirements or control limits.
- **Negative control:** Demonstrates that the agar growth medium is not contaminated during the assay process and consists of pouring uninoculated plates for each lot of media processed.

Incubation and Counting

Incubation provides the appropriate temperature conditions for microorganism growth. The plates are inverted lid-side down to keep condensation from forming on the agar surface. All samples must be aerobically incubated at 32°C. Plates are normally counted at 24, 48, and 72 hours of incubation. The 24- and 48-hour counts provide early information if counts are unexpectedly high, indicating hardware bioburden that may be out of compliance and require CAs. Because of the variable colony morphology, the early counts improve accuracy as colonies may obscure neighboring colonies as they grow. This is illustrated in Figure 34 showing how a 72-hour count might have only eight visible colonies, but the true CFU value is 12 colonies. Early time points can also help identify artifacts or bubbles in the agar that may look like colonies and result in false positives. If there is uncertainty over a spot in or on the agar, at 24 hours or 48 hours it should be marked for further monitoring to see if it changes over time, indicating microbial growth. Any new spots that show up at later time points are likely microbial colonies. For each count performed, record any CFUs. Do not remove the plate covers during incubation or counting to prevent the possibility of airborne contamination. The final CFU count is conducted at 72 hours and represents the final data for each Petri plate.

Figure 34: *Example Colony Counts at 24, 48, and 72 Hours (from NASA-HDBK-6022b)*

Air Sampling Considerations

For air samples, there have been a variety of air monitors used on flight projects. Most work by passing a known volume of air or gas over a collection device, which is usually a plate containing growth media or into a buffered solution to “capture” or

trap bacteria. The monitors include but are not limited to units that employ agar impingement, filter impingement, and direct buffer impingement technologies.

In general, these standard systems capture a fixed volume of air, which can then be directly incubated or processed in a similar fashion to the NSA. The commonly used systems include:

- **Direct Impingement.** Air/sample gas is collected at a fixed velocity and specific time duration onto a Replicate Organism Detection and Counting (RODAC) plate (or a Petri dish), which contains an appropriate agar (growth media) for the organism of interest. Once the sample is collected, the plate is incubated and counted.
- **Direct Gelatin Filter Air Impingement.** Gelatin filter impingement systems collect samples on gelatin membrane filters which can be dissolved into solution or directly plated by placing the filter onto agar. In theory, the advantage of a dissolvable membrane is it can be heat shocked and processed in a manner equivalent to the NSA. The gelatin filters may have some sensitivity to temperature and humidity.
- **Liquid or buffered impingement systems.** Liquid impingement systems concentrate airborne particles in a liquid sample. Air is aspirated at a specific rate and drawn into a liquid cone forming a vortex. Particles are centrifuged into the wall of the cone, thereby separating the particles from air and immersing them in the sample solution. The resulting sample solution can be plated in a similar fashion to the techniques used for the NSA, including heat shock.

Some of the considerations for determining the technique specific for a given mission may include:

1. **Sampling preparation and laboratory resources.** Sample media (agar plates) are subject to contamination and temperature constraints and have a relatively short shelf life.
2. **Turnaround time needed for sample analysis.** Rapid analysis of the samples is not available, as the incubation time for most fungi to form identifiable colonies is typically 5-10 days.
3. **Limits of detection and biological loading.** Plates, gels, and liquids may be sensitive to overloading with viable colonies.
4. **Biological contamination of interest and relationship to spacecraft bioburden assessments.** Air impingement methods may not differentiate between spores and vegetative cells if needed. Liquid air impingement may offer an increased flexibility for sample assessment in that the liquid can be directly plated or used to extract biological molecules (e.g., nucleic acids).
5. **Controls and implementation.** It may be important to differentiate between samples, so controls and cleaning may have to be coordinated. For example, air sampling is used to determine biological loads between a building's various cleanrooms, between cleanrooms and gowning rooms or airlocks, and on supplemental environmental control systems where the inline air conditioning duct is sampled.

Validation and Calibration

The data is expressed in CFU per liter of air. The instruments rely on regulated airflow to capture sample constituents and are dependent on the other electrical mechanisms to work properly. It is therefore suggested that the unit should be validated at least every 6-12 months. The validation of the airflow rate should be done by a certified anemometer in a wind tunnel, and the certification may be performed by the manufacturer or an official distributor. Validation of the system is determining the consistency of the unit in the required environment with an appropriate level of confidence in the data results.

There are several factors that should be considered when implementing air monitoring procedures. Unless the collection media (gel or liquid) can be heat shocked, data output will include colonies from both vegetative microorganisms and spores. The detection limit of the instrument is also relevant, particularly in the analysis of ultra-clean purge gases used near the spacecraft and during launch operations. The sample volume is controlled by the amount of air passed over the collection device during the sample process. The analyst will need to verify that the sample volume is sufficient to capture CFU at the required level. A non-detect reading may be accurate or it may be the function of not enough sample passing through the capture system.

Air monitoring systems are difficult to calibrate directly; calibration requires an air or gas sample containing a known quantity of the organisms of interest. Analysts use a representative environmental sample set to check and determine if the instrument is working properly. Ideally, the environment should mirror the environment to be sampled. The check samples should have

similar CFU profiles over the sample set and should demonstrate that the unit is able to "capture" CFU onto the plate, filter, or liquid interface. During analysis, care should be taken to clean equipment between samples to avoid cross-contamination. Microbiological contamination may occur during the collection, preparation, and analysis of any sample. These interferences should be minimized through the proper use of sterilized equipment, control blanks, and aseptic techniques.

6.1.0 Rapid Biological Contamination Assessments

Rapid biological contamination assessments are useful because the results are obtained quickly without a 72-hour incubation period and can be used to assess if cleaning or other processing is required prior to carrying out and reporting final assays of spacecraft surfaces using the standard spore assay defined in NASA-STD-8719.27 §6. Hardware can be pre-screened for the presence of microbial contamination prior to conducting final assays. NASA has validated total adenosine triphosphate (T-ATP) and the limulus amebocyte lysate (LAL) assays suitable for pre-screening hardware but not bioburden accounting.[112,113]

The T-ATP detection assay is widely used in the food industry to determine gross bacterial contamination. The T-ATP assay uses a bioluminescent reagent containing luciferin and the firefly enzyme luciferase. If present, ATP transfers chemical energy to the luciferin molecule that is then acted upon by the enzyme luciferase (in the presence of magnesium ions and molecular oxygen) to form oxyluciferin, carbon dioxide, and light (bioluminescence). The bioluminescence is then measured with a luminometer using a photomultiplier tube.

For T-ATP, mission data suggests that ATP can be used as a benchmark for cleanliness assessments as long as the hardware has undergone multiple microbial reduction practices and processes (e.g., heat microbial reduction, alcohol cleaning, etc.).[114] Conversely, if hardware has not undergone microbial reduction where the surfaces are not cleaned or maintained clean, the ATP results provide inconsistent cleanliness calls to the NSA. T-ATP values have limited sensitivity to directly detect bacterial spores due to the low levels of ATP found in spores.

LAL is a highly sensitive test developed to detect the presence of pyrogenic endotoxins or lipopolysaccharide (LPS) which may be present on medical devices. The basis of the LAL test is that microbial cell wall material, comprised of LPS, glucan, and peptidoglycans, can trigger a multiple enzymatic cascade which results in the sequential activation of several serine proteases. The assay based on this system can be monitored by quantitative, turbidimetric analysis or by chromogenic or fluorogenic analysis. LAL can be used to measure subpicogram quantities of these microbial products very rapidly with minimal equipment, and can detect live, dead, and non-cultivable organisms. LPS is present only in gram-negative bacteria; the method cannot be easily related to the current pour plate NSA that analyzes spores from microorganisms that are primarily gram-positive. However, because endotoxins are prevalent in nature, the method can be used as a good indicator of the overall biological population (including spores) on flight hardware. Samples are obtained from the field and processed according to manufacturer protocols. The analysis includes an extraction in LAL Reagent Water (LRW) followed by a kinetic chromogenic assay.

Extremes in pH, high concentrations of salts, and the presence of detergents or other organics can interfere with the LAL assay. Each sample is tested "unspiked" or plain and with an LPS "spike" of known concentration added. The measured Endotoxin Units per mL (EU/mL) of the spiked sample when compared to that of the unspiked sample indicates the presence of interference with the LAL assay by the sample. Care should be taken when considering this assay to ensure that all reagents and disposables are endotoxin free.

[112]Venkateswaran, Kasthuri, Noriaki Hattori, Myron T La Duc, and Roger Kern. 2003. "ATP as a Biomarker of Viable Microorganisms in Clean-Room Facilities." *Journal of Microbiological Methods* 52 (3): 367–77.

[113]Wainwright, Norman R, Alice Child, Kendra Williams, Amy Baker, Foster Jordan, Kennda Lynch, and Jud Hedgecock. 2003. "Miniaturized Instrument for Planetary Protection and Life Detection." *SAE Technical Paper Series*, July.

[114]Benardini, James N., and K. Venkateswaran. "Application of the ATP assay to rapidly assess cleanliness of spacecraft surfaces: a path to set a standard for future missions." AMB Express 6 (2016): 113.

6.2.0 PP Sequencing Protocol

This protocol describes a performance-based general procedure for producing DNA sequencing data suitable for satisfying PP requirements. The purpose of this protocol is to provide an outline of required steps and verification methods. It will not provide step-by-step instructions on how to conduct these procedures. A detailed example of an acceptable procedure is available in Stahl-Rommel et al.[115]

Any acceptable protocol must include the following four steps: 1) sample collection, 2) DNA extraction method, 3) DNA sequencing method, and 4) bioinformatics methods to identify organisms present and at least their relative abundance. Each of these steps must include appropriate QA and process validation measures to be acceptable. A validated sequencing procedure demonstrates repeatable results on known samples.[116] Validation steps should be followed before establishing the procedure and repeated after any significant change in the protocol. Initially, it may be necessary to revalidate parts of the procedure each time a new batch of reagents is used. It may be possible to decrease the frequency of revalidation for well-established procedures. QA measures should be used with every sample or set of samples sequenced. Each step is described in greater detail with examples of QA and process validation measures below.

An ideal protocol would produce a result that perfectly replicates the composition of the microbial community present in the sample. Since this is not possible, it is very important to characterize bias introduced by each of these four steps. Biases can be introduced at each step of the process and could include underrepresentation and/or overrepresentation of certain organisms, as well as systematic errors in measurement of the amount of DNA in a sample.[117,118,119,120,121,122] Reagents can also cause bias by adding contaminant DNA to a sample.[123,124] Acceptable protocols should make efforts to minimize bias in the resulting data and should also characterize any bias that is introduced.[125,126,127,128] Unrecognized bias could cause the user to fail to meet PP requirements due to the presence of contaminant DNA in samples or to incorrectly conclude that requirements were met because organisms present in a sample were not detected by the DNA sequencing method.

Sample Collection

Microbiological sampling is the key tool for investigating microbial communities inhabiting an environment. Any biases introduced during sample collection are retained and potentially amplified in every subsequent step. Beyond implementing aseptic techniques, it is critical to standardize the sample collection process to ensure consistency between users and across sampling events. An appropriate method should be able to accommodate the following sample types: surface samples collected with swabs and wipes, liquid samples (e.g., solvents, lubricants, and paints), bioaerosols, and solid samples (e.g., electronics, epoxies, and other types of embedded bioburden). For swabs and wipes, the sampling method should specify sample area, pattern of sampling within the given area, and sampling time. The method should also provide guidance on the pressure to be applied while swabbing or wiping and the area of the sampling tool to be applied to the surface. For liquids and solids, the method should specify a required volume for each sample, acceptable materials, and cleanliness levels for

[115]Stahl-Rommel, S., Jain, M., Nguyen, H. N., Arnold, R. R., Aunon-Chancellor, S. M., Sharp, G. M., et al. (2021). Real-Time Culture-Independent Microbial Profiling Onboard the International Space Station Using Nanopore Sequencing. Genes, 12(1), 106.

[116]Gargis, A. S., Kalman, L., Berry, M. W., Bick, D. P., Dimmock, D. P., Hambuch, T., et al. (2012). Assuring the quality of next-generation sequencing in clinical laboratory practice. Nature Biotechnology, 30(11), 1033–1036.

[117]Hodges, L. R., Rose, L. J., Peterson, A., Noble-Wang, J., & Arduino, M. J. (2006). Evaluation of a macrofoam swab protocol for the recovery of Bacillus anthracis spores from a steel surface. Applied and Environmental Microbiology, 72(6), 4429–30.

[118]Moore, G., Blair, I. S., & Mcdowell, D. A. (2007). Recovery and Transfer of Salmonella Typhimurium from Four Different Domestic Food Contact Surfaces. Journal of Food Protection, 70(10), 2273–2280.

[119]Moore, G., & Griffith, C. (2002). Factors Influencing Recovery of Microorganisms from Surfaces by Use of Traditional Hygiene Swabbing, 22(6), 410–421.

[120]Piepel, G. F., Deatherage Kaiser, B. L., Amidan, B. G., Sydor, M. A., Barrett, C. A., & Hutchison, J. R. (2016). False-negative rate, limit of detection and recovery efficiency performance of a validated macrofoam-swab sampling method for low surface concentrations of Bacillus anthracis Sterne and Bacillus atrophaeus spores. Journal of Applied Microbiology, 121(1), 149–162.

[121]Probst, A., Facius, R., Wirth, R., & Moissl-Eichinger, C. (2010). Validation of a Nylon-Flocked-Swab Protocol for Efficient Recovery of Bacterial Spores from Smooth and Rough Surfaces. Applied and Environmental Microbiology, 76(15), 5148–5158.

[122]Rose, L. J., Noble-Wang, J., & Arduino, M. J. (2015). Surface Sampling. In Manual of Environmental Microbiology (p. 2.6.2-1-2.6.2-14). Washington, DC, USA: ASM Press.OR ; Rose et al., 2011).

[123]de Goffau, M. C., Lager, S., Salter, S. J., Wagner, J., Kronbichler, A., Charnock-Jones, D. S., et al. (2018). Recognizing the reagent microbiome. Nature Microbiology, 3(8), 851–853.

[124]Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., et al. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biology, 12(1), 87.

[125]Davis, N. M., Proctor, D., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2017). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *bioRxiv*, 221499.

[126]Karstens, L., Asquith, M., Davin, S., Fair, D., Gregory, W. T., Wolfe, A. J., et al. (2019). Controlling for Contaminants in Low-Biomass 16S rRNA Gene Sequencing Experiments. *mSystems*, 4(4), e00290-19.

[127]Minich, J. J., Zhu, Q., Janssen, S., Hendrickson, R., Amir, A., Vetter, R., et al. (2018). KatharoSeq Enables High-Throughput Microbiome Analysis from Low-Biomass Samples. *MSystems*, 3(3), e00218-17.

[128]Wright, E. S., & Vetsigian, K. H. (2016). Quality filtering of Illumina index reads mitigates sample cross-talk. *BMC Genomics*, 17, 876.

sample containers. The method should also include instructions on how to collect a representative sample, and whether preservatives or neutralizing buffers are required. Similarly, for bioaerosols, the collection method should describe how to collect a representative sample for the environment (sampler placement), sample duration, and sample volume. The method should also describe required sterilization of the sampler between events and how to validate the sterilization. For all sample types, storage conditions of the sample between collection and analysis must be determined and strictly followed to avoid unwanted degradation or alteration of the microbial community.

Process Validation:

- Determine the analytical sensitivity and limit of detection of the collection method by measuring the number or concentration of cells collected from a standard or reference material with a known composition and concentration of microbes.
- Ensure that the collection method does not preferentially select for certain types of organisms or metabolic states (e.g., spore-forming bacteria or vegetative cells).

QA Steps:

- Sample blanks (10% of the total number of samples) should be included with each sampling event to estimate contamination arising from sampling, sample technique, and storage prior to analysis. In instances where fewer than 20 samples are collected, at least two blanks should also be analyzed. These sample blanks should be handled with the same procedures as those used to collect a sample, except that the blank should not contact the sampled environment. For example, at least two swabs that have never touched an environmental surface should be collected and processed from each sampling event utilizing swabs.

DNA Extraction

An appropriate method must be able to extract DNA from a variety of samples encountered in PP efforts. These sample types should include surface samples collected with swabs and wipes in clean rooms, liquid samples (e.g., solvents, lubricants, and paints), bioaerosols, and solid samples (e.g., electronics, epoxies, and other types of embedded bioburden). Extracting or lysing the cells directly from the sample is preferred. Direct extraction (i.e., extracting DNA directly from the sample material) reduces a source of bias introduced when cells are rinsed or sonicated into a liquid media prior to extraction. The DNA extraction method should be optimized to work with low biomass samples (≤ 1,000 cells per sample) since most samples relevant to PP contain very few cells. The DNA extraction method should be capable of producing a high enough concentration of DNA so that a commercial or academic core facility can sequence the sample as part of their routine operations. Any biases introduced during library preparation should be well characterized.

Process Validation:

- Ensure extraction preserves diversity in the mock communities/reference samples.
- Ensure extraction yield is high and replicable from positive controls containing known amounts of DNA (mock community/reference sample should work for this).[129]
- Ensure spike in controls or internal standards are measurable in negative controls. Use these values to set the analytical sensitivity and limit of detection for the method. Spike in controls should consist of known concentrations of organisms not routinely encountered in cleanroom samples.
- Extract replicates from mock communities and reference samples multiple times to establish thresholds for extraction repeatability and precision.

QA Steps:

- Extract positive controls
  - Mock community (reference sample)
  - Low biomass mock community (reference sample)
- Extract negative controls
  - Reagent blank
  - Sample blank
  - Matrix blank

[129]Caruso, V., Song, X., Asquith, M., & Karstens, L. (2019). Performance of Microbiome Sequence Inference Methods in Environments with Varying Biomass. *mSystems*, 4(1), e00163-18.

- Extract spike in controls
  - Add spike in controls to samples and negative controls
  - Spike in controls should consist of known concentrations of organisms not routinely encountered in cleanroom samples

DNA Sequencing

The DNA sequencing method must be able to accurately sequence DNA from bacteria, archaea, fungi, and other eukaryotes. The sequencing method should produce data in a standard file format with quality scores for each base pair (e.g., FASTQ files). Biases in the type or quantity of DNA sequenced should be well characterized. For example, some DNA sequencers struggle to accurately sequence homopolymers (DNA sequences with long stretches of a single nucleotide). DNA sequencing bias should be well characterized by repeatedly running the appropriate control samples.

Process Validation:

- Sequencing positive controls should produce consistent results from different sequencing runs and different reagent batches.
- Ensure sequencing preserves diversity in the mock communities.
- Characterize GC bias induced by the sequencer.
- Ensure spike in controls are correctly sequenced in all samples where they are added. Use these values to set the analytical sensitivity and the limit of detection for the method (see Table 10 below for definitions). Spike in controls should consist of known concentrations of organisms not routinely encountered in cleanroom samples.
- Characterize “kit contaminant” sequences in negative controls.
- Establish a baseline depth of coverage (the number of times a nucleotide sequence is read by the sequencer) necessary to accurately sequence reference samples.
- Sequence mock communities and reference samples multiple times to set acceptable repeatability and precision thresholds.
- Sequence controls, blanks, and reference samples on a different sequencer (orthogonal testing) to characterize bias of the chosen sequencing platform.

QA Steps:

- Sequence positive controls
  - Mock community
  - Low biomass mock community
- Sequence negative controls
  - Reagent blank
  - Sample blank
  - DNA extraction matrix blank
  - Library preparation blank
- Sequence spike in controls
  - Add spike in controls to samples and negative controls
  - Spike in controls should consist of known concentrations of organisms not routinely encountered in cleanroom samples

Bioinformatics

Bioinformatics pipelines serve dual purposes. They should be able to filter out low-quality sequences and samples with little or no sequenceable DNA. They should also align high-quality sequences to a reference database and identify the type and relative or absolute abundance of organisms present in a sample.[130] Output from bioinformatics pipelines should allow the user to determine if a sample is clean enough to meet PP requirements or if additional bioburden reduction is required. As an alternate method to meet PP requirements, aligned sequences could be compared to a database containing organisms that are particularly well suited to survive conditions on the target body or genes that may confer enhanced survival at the target body.[131] Alpha diversity or species richness (number of different organisms) could also be used as a risk assessment metric, where samples with a higher diversity of organisms present are assumed to be less clean than samples with lower diversity.

[130]Gargis, A. S., Kalman, L., Bick, D. P., da Silva, C., Dimmock, D. P., Funke, B. H., et al. (2015). Good laboratory practice for clinical next-generation sequencing informatics pipelines. Nature Biotechnology, 33(7), 689–693.

[131]Singh, N. K., Arora, N., Cooper, M., & Mahabal, A. (2022). Assessment of contamination potential of Spacecraft surface microbes using the metagenomic data. In *44th COSPAR Scientific Assembly.* (Vol. 44, p. 3275). Athens Greece. Retrieved from **https://ui.adsabs.harvard.edu/abs/2022cosp...44.3275S**

Process Validation:

- Bioinformatics pipelines should be validated separately from the extraction and sequencing methods with externally validated data sets.
- Ensure sequences from mock communities, reference samples, and spike in controls align to the correct sequences in the reference database.

QA Steps:

- Sequences carried forward for further analysis should have <1E-3 probability of being incorrect (Phred quality score ≥30).
- Samples should have a minimum number of reads to be used in downstream analysis. Sequencing negative controls can help determine the threshold for each sequencing run.
- Organisms of PP interest/concern should be identifiable with multiple bioinformatics tools. If a bioinformatics tool does not identify an organism of concern known to be in a control sample, it should not be used.
- Ensure that positive controls, reference samples, and spike in controls contain the correct sequences or organisms and that relative abundance in mock communities is preserved.
- Ensure the tools are capable of identifying organisms at the appropriate taxonomic level (e.g., genus, species, or strain identifications).

Table 10: *Definitions of Validation Criteria That Should Be Considered for Any Method*[132,133,134]

Criteria	Definition
Extraction Efficiency	Amount of DNA extracted from a sample relative to the known amount of DNA added to a sample.
Accuracy	The degree of agreement between the nucleic acid sequences derived from the assay and a reference sequence.[135] $\frac{(TP + TN)}{(TP + FP + FN + TN)}$
Precision	The degree to which repeated sequence analyses give the same result repeatability (within-run precision) and reproducibility (between-run precision).
Analytical Sensitivity	The likelihood that the assay will detect the targeted sequence variations, if present. $\frac{TP}{(TP + FN)}$
Analytical Specificity	The probability that the assay will not detect a sequence variation when none are present (the false positive rate is a useful measurement for sequencing assays). $\frac{TN}{(TN + FP)}$
Reproducibility	The degree to which the same result(s) is obtained for a sample when the assay is repeated between/among different operators and/or detection instruments.
Repeatability	The degree to which the same result(s) is obtained for a sample when the assay is repeated by the same operator and/or detection instrument.
False Positive Rate	The rate at which a target is incorrectly called as present. Also known as Type I error. Calculated as 1 – specificity.
False Negative Rate	The rate at which a target organism is incorrectly called as absent. Also known as Type II error. Calculated as 1 – sensitivity.
Limit of Detection	Minimum level of input material for a target as a proportion of the total at which all replicates are consistently positive for that target.

[132]Budowle, B., Connell, N. D., Bielecka-Oder, A., Colwell, R. R., Corbett, C. R., Fletcher, J., et al. (2014). Validation of high throughput sequencing and microbial forensics applications. I*nvestigative Genetics*, 5(1), 9.

[133]Gargis et al., 2012

[134]U.S. Food and Drug Administration Foods Program. (2020). Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products (No. Edition 1.1). Retrieved from **https://www.fda.gov/media/121751/download**

[135]TP = True Positive, TN = True Negative, FP = False Positive, FN = False Negative

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7.0.0 Biological Estimation Techniques

Navigation Summary: *Chapter 7 discusses the accounting for organic materials present on the spacecraft, archiving of bulk organic materials present on the spacecraft, and assessment of spacecraft bioburden. This chapter is applicable to Category II, III, IV, and V missions.*

Once a swab or wipe sample is collected from a spacecraft or spacecraft-associated surface, the standard spore assays yield a spore count for each sample. These spore counts can be statistically treated to calculate a bioburden density for a given component, typically expressed as a total number of microorganisms per unit area (i.e., CFU/m^2). A bioburden density estimate should factor in the following variables: (1) observed CFU(s); (2) representative volume processed; (3) sampled surface area; (4) sampling device efficiencies; (5) sample processing efficiencies; and (6) laboratory efficiencies for a given standard operating protocol/user.

There have been several recognized bioburden accounting techniques that were implemented by missions since the Mars Viking Missions to include a sum-of-the-means approach and Poisson and Gaussian statistics.[136] In addition to these accounting techniques, a Bayesian framework may allow end users to better describe bioburden estimate ranges and uncertainty in the sampling, processing, and spacecraft build process to better manage a project's risk posture.[137] Typically, the datasets that are collected from spacecraft hardware are zero-inflated datasets with over 85% of the samples yielding a zero-spore count. Factoring in a zero-inflated dataset should be assessed as the project is selecting the bioburden accounting technique. The mission should propose the biological estimation technique, assumptions, and rationale in the mission's PPIP.

7.1.0 Organic Inventory, Biological Inventory, and Organic Archiving

Scientific exploration activities searching for extraterrestrial life, or the evidence of extraterrestrial life, and studying prebiotic chemistry in the solar system are sensitive to sources of molecular and biological contamination. Forward PP seeks to control the harmful contamination of solar system bodies by terrestrial organisms, organic materials, and organic volatile materials carried on or released by spacecraft during space exploration operations to preserve the ability to perform these scientific studies for current and future missions. Organic inventory, biological inventory, and organic archiving requirements provided in Section 4.3 of NASA-STD-8719.27 address potential sources of molecular and biological contamination introduced by spacecraft during solar system exploration.

The terms *organic inventory, biological inventory*, and *organic archive* refer to specific deliverables required by mission providers as defined in NASA-STD-8719.27. *Organic inventory* refers to the list of organic materials present on a spacecraft in amounts greater than 1.0 kg for PP Category II, IIb (Lunar), III, and IV missions. Furthermore, the organic inventory for missions to Earth's Moon have separate requirements and are limited to organic materials released by propulsion and attitude control systems for PP Category IIa and IIb missions. These requirements stem from COSPAR PP guidelines.[138]

Biological inventory refers to estimates of all biological materials such as intact organisms, remnants of organisms, and biological molecules intentionally included as part of the hardware elements to reach the target solar system body for a PP Category III or IV mission. These include biological materials present on any payload, spacecraft, launch vehicle, or descent stage. However, this does not include biological materials present on or in the spacecraft as a result of the assembly and integration process. The focus of the biological inventory is the biological materials that are intentionally included. For example, if a mission were to propose transporting seeds for biological experiments on Mars, the seeds are included in the biological inventory. An estimate of the number of human skin cells deposited on hardware surfaces as a result of cleanroom particle fallout would not be included in the biological inventory.

Organic archive refers to the process of archiving a sample of all organic materials that are present on a spacecraft in amounts greater than 25 kg and are present through the end of the mission for PP Category III and IV missions. Archived material samples should be representative of flight hardware and collected from those materials processed for flight.

[136] J. Nick Benardini, Arman Seuylemezian, and Andrei Gribok. 2020. "Predicting Biological Cleanliness: An Empirical Bayes Approach for Spacecraft Bioburden Accounting." *OSTI OAI (U.S. Department of Energy Office of Scientific and Technical Information)*, March.

[137] Gribok, Andrei, Arman Seuylemezian, and James Benardini. 2021. "Application of a Bayesian Statistical Framework for Planetary Protection as a Means of Verifying Low-Biomass, Zero-Inflated Test Data from Spacecraft." *Life Sciences in Space Research* 30 (August): 39–44.

[138] A. Coustenis, Niklas Hedman, Peter T Doran, Omar Al Shehhi, E. Ammannito, M Fujimoto, O. Grasset, et al. 2023. "Planetary Protection: An International Concern and Responsibility." *Frontiers in Astronomy and Space Sciences* 10 (May).

These materials should have experienced the same material processes as the flight hardware, including machining, curing, chemical treatments, bakeouts, etc.

It should be noted that the terms *inventory* and *archive* have been used interchangeably over time, creating confusion amongst PP community practitioners. NASA-STD-8719.27 has attempted to alleviate some of this confusion by defining an inventory as a list of materials, and an archive as a collection of stored physical material samples.

7.1.1 Historical Background of Requirements

The concern for accounting for organic materials of terrestrial origin stems from the early lunar exploration programs of the 1960s. A planning study for creating an organic constituent inventory program in 1968 cited trace constituents in 1 kg of organic material samples as a contamination concern for investigators analyzing lunar samples for precursors to life.[139] Later, the focus shifted from contamination of the lunar regolith to the water ice present at the lunar poles and PSRs. A 2020 NASEM study on PP for lunar volatiles concluded an organic inventory of materials carried on spacecraft for PP Category II missions to Earth's Moon should be required to support studies of "the evolution of biologically relevant chemical compounds."[140]

The concerns of terrestrial contamination that existed in lunar mission design were carried forward to the exploration of Mars. The Viking Program required an organic inventory of bulk (>1 kg) organic constituents of all launched hardware that would directly contact Mars in the case of normal mission operations and in the case of an unplanned impact. Viking also introduced the requirement for an organic archive of 50 gram samples of each organic compound whose total amount in the landing system exceeds 25 kg to be stored for 15-20 years or longer.[141] Organic archiving of material samples has occurred for each Mars mission.[142]

7.1.2 Reporting of Inventories and Archives

Mission providers have the flexibility to choose any reporting format for organic inventories and archives. Generation of organic inventories can occur in parallel with similar activities for identifying materials for materials and process engineering (M&PE) and CC efforts, such as generation of materials identification and usage lists (MIULs) or material lists for input into spacecraft outgassing analysis. Alternatively, a simple template as provided in *Appendix 3: PP Category II Mission Organic Inventory Template* may be completed. All inventories and archives are captured as part of the PP Pre-Launch Report. Any changes in information submitted from the PP Pre-Launch Report should be captured in the PP Post-Launch Report or PP End-of-Mission Report.

7.1.3 Current Limitations, Challenges, and Opportunities for Improvement

The PP requirements for organic inventories and archiving have not changed since the early lunar and Viking missions. Limitations and challenges to organic inventories and archiving were identified over the decades that mission providers have been satisfying reporting requirements. Some of these limitations and challenges identified include, but are not limited to:

- The organic inventory is a list identifying each material by commercial name and an estimate of the material mass. This list does not provide the detailed history and processing of each organic material (e.g., processing, conditioning, handling, and cleaning), which can greatly affect the volatile organic content of the material.

[139]Lyle, Robert G. TRSR-68-029: "Planning Study for an Organic Constituents Inventory Program," Exotech Inc., 1968, **https://ntrs.nasa.gov/api/citations/19680018443/downloads/19680018443.pdf.**
[140]Report Series: Committee on Planetary Protection. 2020. National Academies Press EBooks.
[141]Werber, Morton. NASA SP-344: "Objectives and Models of the Planetary Quarantine Program," NASA Headquarters, Washington, DC, 1975. **https://ntrs.nasa.gov/api/citations/19750017532/downloads/19750017532.pdf**.
[142]**https://planetaryprotection.jpl.nasa.gov/biological-materials-archive**

- Material manufacturing and conditioning history can be difficult and sometimes impossible to obtain, as many suppliers consider the information to be intellectual property and classify it as a proprietary or trade secret.
- Identifying and listing bulk organic materials do not inform on the potential contaminants that may enter an environment. A bulk organic materials list does not account for contaminant mixing, reactions, or mitigation activities such hermetic seals and thermal bakeouts that may greatly reduce the potential for contaminants to reach an environment.
- The potential contribution of organic molecular contamination from spacecraft materials cannot be determined from an organic material inventory alone, and additional modeling and analysis is required to determine contamination risks and impacts to science.
- Archived materials may not be stable for long-term storage or may change in ways that are different from space-exposed materials.
- The rationale for providing an organic inventory and archive is based on a reactive position of attempting to determine sources of contamination after a problem has occurred, rather than a proactive position of addressing the specific needs of science. Mission providers expend resources to create inventories and archives for potential problems that may not occur.

Given these limitations and challenges, opportunities exist to explore potential changes in PP requirements and reporting based on new research and technology developments and capabilities. Some of these opportunities include, but are not limited to:

- Utilizing analysis knowledge and modeling capability developed by the CC community to apply volatile organic molecular contamination analysis to PP issues. The analysis performed by CC engineers is often verified through thermal vacuum bakeouts of spaceflight hardware and outgassing tests in chambers instrumented with quartz crystal microbalances, vacuum gauges, and residual gas analyzers to identify chemical "fingerprints" of contaminants. This information may inform PP contamination risk analyses of target bodies.
- Exploring a NASEM finding from a lunar volatiles study for PP, which found a "lack of, and need for, studies to characterize the chemical composition, transport, and the level of contamination of volatiles that would be harmful to future investigations of prebiotic chemical evolution to be pursued at PSRs. This information is necessary to determine whether to establish PP requirements for missions to these areas of the Moon, such as a requirement for reporting the inventory of propellants, combustion products, and potential off-gassing volatiles from spacecraft."[143] Such a study would provide rationale for maintaining an organic inventory, or for exploring other methods of characterizing organic contamination.
- Holding workshops with the science and engineering communities to explore the requirements trade space to inform future requirements updates and changes.

7.3.0 Bioburden Accounting Parameters

In missions using a bioburden approach, there are often some surfaces or volumes that are not able to be sampled for various reasons. In these cases, conservative standard bioburden densities can be assigned based on type of material and manufacturing environment to contribute to the Current Best Estimate (CBE) of the total bioburden at launch. The application of these standard bioburden densities should be detailed in the PPEL or bioburden accounting tool. NASA-STD-8719.27 Appendix C specifies accepted bioburden densities for encapsulated bioburden (microorganisms that might be trapped within the volume of a substance) and expected densities based on cleanroom environment. Bioburden on unsampleable surfaces can be further reduced by applying approved bioburden reduction treatment such as Heat Microbial Reduction (HMR) or VHP.[144,145]

[143]Report Series: Committee on Planetary Protection. 2020. National Academies Press EBooks. **https://doi.org/10.17226/26029**.
[144]ECSS-Q-ST-70-57C 30 August 2013: **https://ecss.nl/standard/ecss-q-st-70-57c-dry-heat-bioburden-reduction-for-flight-hardware-30-august-2013/**
[145]ECSS-Q-ST-70-56C 30 August 2013: **https://ecss.nl/standard/ecss-q-st-70-56c-vapour-phase-bioburden-reduction-for-flight-hardware-30-august-2013/**

8.0.0 Backward PP

Navigation Summary: *Chapter 8 discusses the implementation of Category V Earth return missions, particularly restricted Earth return missions, V(r). This chapter is applicable to Category V missions.*

The purpose of backward PP is to prevent harmful biological contamination of the Earth-Moon system by potential extraterrestrial life and bioactive molecules in returned samples and spacecraft from sensitive solar system bodies. The intent behind calling out the Earth-Moon system specifically is two-fold: 1.) to capture the primary goal of preventing adverse impacts to Earth's biosphere, and 2.) to recognize the importance of not bringing extraterrestrial life or bioactive molecules to the Moon. Given humanity's interest in increased lunar exploration and utilization, it is important that return materials do not adversely impact ongoing activities on the Moon. Sensitive solar system bodies (e.g., Mars, Europa, and Enceladus) are designated as a Category V restricted V(r) Earth return mission. All other solar system bodies designated as Category V are unrestricted V(u) Earth return missions. Unrestricted Earth return missions do not require further PP consideration for the return phase of the mission. Restricted Earth return missions must consider controls to prevent harmful contamination prior to Earth entry, during Earth entry, and during Earth containment.

The consequences of backward contamination are the potential proliferation of life or bioactive molecules resulting in adverse changes to the Earth's biological environment. Scientific consensus has defined the potential life or bioactive molecules as Earth-based biology (life as we know it). Scientists also include a broad spectrum of bioactive molecules, including bacteria, spore formers, archaea, fungi, viruses, prions, plasmids, genetic transfer agents, etc.[146,147,148] Back PP risk assessments have considered the likelihood of arrival on Earth of uncontained, unsterilized material from solar system bodies by natural interplanetary transit.[149,150] Adequately assessing risk and determining the best mitigation path is perhaps one of the most important activities for PP mission success for a sample return mission.

Apollo 11, 12, and 14 were the only Missions to date that were implemented with requirements that parallel what would now be known as a Category V Restricted Earth return mission.[151] The restricted Earth return implementation for those Missions applied to both the crew and the return samples. An Interagency Advisory Committee on Back Contamination (ICBC) supervised NASA's implementation planning and operations. Some of the activities included building and commissioning a state-of-the-art lunar receiving and crew quarantine facility, cleaning the exterior of the capsule with disinfectants, crew quarantine, and sample safety assessments to determine that the samples were safe for public release.

The authorization to bring Category V restricted Earth return samples back to Earth is governed by the 1977 Presidential Directive/National Security Council (PD/NSC)-25,[152] *Scientific or Technological Experiments with Possible Large-Scale Adverse Environmental Effects and Launch of Nuclear Systems into Space*. PD/NSC-25 directs NASA, as the sponsoring agency, to prepare a detailed evaluation of the possible environmental effects for the Director of the Office of Science and Technology Policy, who may request additional studies, consultations with other U.S. Government or intergovernmental/ international bodies, and Presidential approval.

Prevention of backward contamination can be achieved through a series of accepted controls that may include sterilization, isolation and containment of samples and hardware, and conducting a sample safety assessment to determine if the samples are safe to release. Notably, for crewed missions, the return samples, hardware, and crew should be considered in developing a public safety and biosphere protection strategy. Further details are provided in this chapter of the handbook to address these controls and considerations for implementing back PP on a restricted Earth return mission.

[146]Amman, Walter, John Barros, Allan Bennett, Jim Bridges, Joseph Fragola, Armel Kerrest, Nicolas Walter, et al. 2012. "Mars Sample Return backward contamination – Strategic advice and requirements." Report from the ESF-ESSC Study Group on MSR Planetary Protection Requirements.

[147]Craven, Emily, Martell Winters, Alvin L. Smith, Erin Lalime, Rocco Mancinelli, Brian Shirey, Wayne Schubert, et al. 2021. "Biological Safety in the Context of Backward Planetary Protection and Mars Sample Return: Conclusions from the Sterilization Working Group." *International Journal of Astrobiology* 20 (1): 1–28.

[148]Gerhard Kminek, James N Benardini, Frank E Brenker, Timothy Brooks, Aaron S Burton, Suresh Dhaniyala, Jason P Dworkin, et al. 2022. "COSPAR Sample Safety Assessment Framework (SSAF)." *Astrobiology* 22 (S1): S-216.

[149]"Mars Sample Return." 1997, February. **https://doi.org/10.17226/5563**.

[150]Assessment of Planetary Protection Requirements for Mars Sample Return Missions. 2009. National Academies Press EBooks. **https://doi.org/10.17226/12576**.

[151]Meltzer, Michael. 2011. *When Biospheres Collide: A History of NASA's Planetary Protection Program*. NASA SP-2011-4234.

[152]Presidential Directive PD/NSC-25 (1977) Scientific or Technological Experiments with Possible Large-scale Adverse Environmental Effects and Launch of Nuclear Systems into Space, Washington DC. Accessible at: **https://www.jimmycarterlibrary.gov/sites/default/files/pdf_documents/assets/documents/directives/pd25.pdf**

8.1.0 Sample Safety Assessment Protocol

Missions returning samples or spacecraft from sensitive solar system bodies are required to consider either an accepted sterilization/inactivation process or perform a sample safety assessment on each sample prior to release from containment. Both processes need to incorporate updated scientific consensus. Approval for sample release should be coordinated as part of the Presidential Directive (PD)/NSC-25[153] process and agreed upon within the U.S. Government interagency and international communities.

The sample safety assessment for each sample is performed in containment and has a long historical background dating back to Apollo. For example, the Apollo samples underwent a sample safety assessment where Moon regolith was used to attempt to culture microorganisms on agar Petri dishes, fed to an assortment of animals, and used as a supplement for a variety of plants.[154] Leveraging these lessons learned throughout the decades and with our expanded knowledge of Earth's biosphere, a statistical framework was recently proposed by the international community that seeks to look at the fundamental tenets of life.[155,156,157,158,159] This framework does not necessarily address the safety of the sample, but does provide context about how one would establish a detailed protocol and decision-making process to determine if life is present or absent in the sample. If life is absent, then the sample is considered safe for release. Briefly, this framework focuses on a test sequence that considers the following for each sample: three-dimensional structural investigation, gas analysis, chemistry and mineralogy associated with the three-dimensional investigation, organic molecule analysis, characterization of molecular patterns (particularly organic-rich material), a targeted polymeric or large molecule search to differentiate from abiotic macromolecules, and an evaluation of life as we know it.

Sterilization of the sample using a scientifically agreed-upon process before release is another option. If a sample is sterilized using that process, it does not need to undergo a sample safety assessment protocol. Developing an appropriate sterilization process validation should be considered. While a sterilization process has not been formalized to date, the scientific community has considered dry heat and g-irradiation as contenders.[160]

8.2.0 PP Knowledge Gaps for Crewed Missions to Mars

As we prepare for the first mission to Mars with a human crew, we have a continuing obligation to protect against harmful contamination at the Red Planet. In particular, it is unlikely that the search for life on Mars will be completed by the time the first crew systems arrive at the Martian surface. Indeed, some consider the presence of astronauts to be an essential augmentation to the robotic search for evidence of life. In addition, the environment of the Earth needs to be protected from the threat posed by the uncontrolled release of a putative Martian life form into the terrestrial biosphere.

Prevention of such harmful cross-contamination between Mars and the Earth is the practice of PP. At present, the knowledge of how to achieve these two goals (prevention of forward contamination from Earth and backward contamination from Mars) is well described for robotic systems. In contrast, for human missions, there are guidelines[161] that are insufficient to guide engineering design, in part because our knowledge of Mars (and of how contamination from crewed systems will interact with Mars) is incomplete. These gaps in our knowledge need to be addressed by acquiring new data during the next decade if PP measures are to be implemented successfully for human missions.

The process to develop requirements for PP for a crewed mission to Mars includes holding a series of meetings with an international group of multidisciplinary scientists and engineers to address PP issues for human missions to Mars. These

[153] See Footnote 151.

[154] **https://ntrs.nasa.gov/api/citations/19680021536/downloads/19680021536.pdf**

[155] Gerhard Kminek, James N Benardini, Frank E Brenker, Timothy Brooks, Aaron S Burton, Suresh Dhaniyala, Jason P Dworkin, et al. 2022. "COSPAR Sample Safety Assessment Framework (SSAF)." Astrobiology 22 (S1): S-216.

[156] **https://nap.nationalacademies.org/catalog/5563/mars-sample-return-issues-and-recommendations**

[157] Devincenzi, Donald, and John Rummel. 1999. "MARS SAMPLE QUARANTINE PROTOCOL WORKSHOP." **https://ntrs.nasa.gov/api/citations/19990087432/downloads/19990087432.pdf**.

[158] The Quarantine and Certification of Martian Samples. 2002. Washington, D.C.: National Academies Press. **https://doi.org/10.17226/10138**.

[159] Rummel, John, Headquarters Washington, D Margaret, S Race, Donald Devincenzi, P Jackson, Schad Nasa, D Pericles, D Stabekis, and Sara Acevedo. 2002. "A DRAFT TEST PROTOCOL for DETECTING POSSIBLE BIOHAZARDS IN." **https://ntrs.nasa.gov/api/citations/20030053046/downloads/20030053046.pdf**.

[160] Meÿer, Michael A, Gerhard Kminek, D W Beaty, Brandi Carrier, T. Haltigin, L E Hays, C B Agee, et al. 2022. "Final Report of the Mars Sample Return Science Planning Group 2 (MSPG2)." Astrobiology 22 (S1): S-26.

[161] See Footnote 23.

meetings started with a NASA-only meeting in 2015, leaning on earlier considerations of the topic to identify knowledge gaps (KGs) that would need to be addressed to develop engineering requirements for PP for crewed missions to Mars. Since 2015, the topic of PP KGs for human missions has been systematically addressed during a meeting series led by COSPAR, but co-sponsored by NASA and the European Space Agency (ESA). These were open meetings, typically comprised of 50-60 in-person attendees (later virtual meetings were larger at 80-100), and at various times the meeting attendees included spacecraft engineers, scientific discipline specialists, astronauts, legal professionals, crew flight surgeons, program and project managers, and representatives of commercial spaceflight organizations.

The KGs that were generated were grouped into three study areas:

1. Microbial and human health monitoring
2. Technology and operations for contamination control (also called “spacecraft systems”)
3. Natural transport of contamination on Mars

Closure of these KGs would lead to an end-to-end knowledge-based solution for countries and organizations seeking to comply with the Operations Support Team to set PP requirements and develop implementation procedures for the first human mission to Mars. The workshop series and its findings are described in more detail in the Spry et al paper, “Planetary Protection Knowledge Gap Closure Enabling Crewed Missions to Mars,” accepted for publication in *Astrobiology* in 2024.[162]

[162]J Andy Spry, Bette Siegel, Gerhard Kminek, Corien Bakermans, J. Nick Benardini, Esther Beltran, Rosalba Bonaccorsi, et al. 2021. “Planetary Protection Knowledge Gaps and Enabling Science for Human Mars Missions” Bulletin of the AAS. 53 (4).

Appendix 1: Acronyms

AIHA	American Industrial Hygiene Association
APL	Applied Physics Laboratory
ASTM	American Society for Testing and Materials
ATLO	Assembly, test, and launch operations
ATP	Adenosine Triphosphate
BAT	Best Available Technology
BSC	Biological safety cabinet
CBE	Current Best Estimate
CC	Contamination Control
CCAM	Contamination and collision avoidance maneuvers
CDC	Centers for Disease Control and Prevention
CDF	Cumulative density function
CDR	Critical Design Review
CFU	Colony Forming Units
CoPP	Committee on Planetary Protection
COSPAR	Committee on Space Research
CRM	Certified Reference Material
DDL	Deorbit, Descent, and Landing
DHMR	Dry heat microbial reduction
DQO	Data Quality Objective
EDL	Entry, descent, and landing
EPA	Environmental Protection Agency
ESA	European Space Agency
ESD	Electrostatic discharge
EtO	Ethylene oxide
EU	Endotoxin Units
FDA	Food and Drug Administration
FFRDC	Federally Funded Research and Development Center
FMEA	Failure mode and effect analysis
FN	False Negative
FOD	Foreign object debris
FP	False Positive
FTA	Fault tree analysis
GCR	Galactic Cosmic Rays
GSE	Ground support equipment
HACCP	Hazard analysis critical control point
HEPA	High-efficiency particulate air
HMR	Heat Microbial Reduction
ICBC	Interagency Advisory Committee on Back Contamination
ICM	Injection covariance matrix
IPA	Isopropyl Alcohol
IPM	Integrated pest management
ISA	Incident Surprise Anomaly
ISO	International Organization for Standardization
JIMO	Jupiter Icy Moons Orbiter
JOI	Jupiter orbit insertion
JPL	Jet Propulsion Laboratory
KG	Knowledge gap
KSC	Kennedy Space Center
LAL	Limulus amebocyte lysate
LEAG	Lunar Exploration Analysis Group
LPS	Lipopolysaccharide
LRW	LAL Reagent Water
M&PE	Materials and process engineering
MCMC	Markov Chain Monte Carlo
MDAA	Mission Directorate Associate Administrator
MEPAG	Mars Exploration Program Analysis Group
MIUL	Material usage and information list
MOMA	Mars Organic Molecule Analyzer
NASA	National Aeronautics and Space Administration
NASEM	National Academies for Science, Engineering, and Medicine (or National Academies)
NPR	NASA Procedural Requirements
NSA	NASA Standard Assay
NSC	NASA Safety Center
OPAG	Outer Planets Assessment Group
OPP	Office of Planetary Protection
OSMA	Office of Safety and Mission Assurance
OST	Outer Space Treaty
PD/NSC	Presidential Directive/National Security Council
PDF	Probability density function
PDR	Preliminary Design Review
PFR	Performance/Failure anomaly Report
PHSF	Payload Hazardous Servicing Facility
PP	Planetary Protection
PPE	Personal protective equipment
PPEL	Planetary Protection Equipment List
PPP	Panel on Planetary Protection
PRA	Probabilistic Risk Assessment
PREVCOM	Preventing the Forward Contamination of Mars
PSE	Project Systems Engineering
PSR	Permanently shadowed region
PT	Proficiency testing
QA	Quality Assurance
QC	Quality Control
RIDM	Risk-informed decision-making
RODAC	Replicate Organism Detection and Counting
RQ	Requirement
SATERN	System for Administration, Training, and Educational Resources for NASA
SCARF	Spacecraft Assembly and Rotation Fixture

SIR	System Integration Review
SMD	Science Mission Directorate
SME	Subject matter experts
SOI	Sphere of influence
SOP	Standard operating procedure
SRP	Solar radiation pressure
SRR	System Requirements Review
SR-SAG	Special Regions – Science Analysis Group
TA	Technical Authority
T-ATP	Total adenosine triphosphate
TCM	Trajectory correction maneuver
TNC	Too Numerous to Count
TN	True Negative
TP	True Positive
TSA	Tryptic soy agar
UNOOSA	United Nations Office for Outer Space Affairs
UQ	Uncertainty Quantification
UV	Ultraviolet
UV-C	Ultraviolet-C
V&V	Verification and Validation
VHP	Vapor hydrogen peroxide
V(u)	Category V unrestricted Earth return
V(r)	Category V restricted Earth return
VTO	Viable terrestrial organism

Appendix 2: National Academies Reports

Year	Title	General	Mars	Icy Worlds	Other	Text	Citation
1965	Conference on Hazard of Planetary Contamination Due to Microbiological Contamination in the Interior of Spacecraft Components	X				https://nap.nationalacademies.org/read/12414	National Research Council. 1965. Conference on Hazard of Planetary Contamination Due to Microbiological Contamination in the Interior of Spacecraft Components. Washington, DC: The National Academies Press. https://doi.org/10.17226/12412.
1967	Report of Panel Study on the Biological Quarantine of Venus				X	https://nap.nationalacademies.org/read/25589	National Research Council. 1967. Report of Panel Study on the Biological Quarantine of Venus. Washington, DC: The National Academies Press. https://doi.org/10.17226/25589.
1972	Review of Planetary Quarantine Policy	X				https://nap.nationalacademies.org/read/12388	National Research Council. 1972. Review of Planetary Quarantine Policy. Washington, DC: The National Academies Press. https://doi.org/10.17226/12388.
1978	Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan	X				https://nap.nationalacademies.org/read/20034	National Research Council. 1978. Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan. Washington, DC: The National Academies Press. https://doi.org/10.17226/20034.
1985	On NASA Policy for Planetary Protection: Letter Report	X				https://nap.nationalacademies.org/read/12359	National Research Council. 1985. On NASA Policy for Planetary Protection: Letter Report. Washington, DC: The National Academies Press. https://doi.org/10.17226/12359.
1992	Biological Contamination of Mars: Issues and Recommendations		X			https://nap.nationalacademies.org/read/12305	National Research Council. 1992. Biological Contamination of Mars: Issues and Recommendations. Washington, DC: The National Academies Press. https://doi.org/10.17226/12305.
1997	Mars Sample Return: Issues and Recommendations		X			https://nap.nationalacademies.org/read/5563	National Research Council. 1997. Mars Sample Return: Issues and Recommendations. Washington, DC: The National Academies Press. https://doi.org/10.17226/5563.
1998	Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies: Framework for Decision Making				X	https://nap.nationalacademies.org/read/6281	National Research Council. 1998. Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies: Framework for Decision Making. Washington, DC: The National Academies Press. https://doi.org/10.17226/6281.
2000	Preventing the Forward Contamination of Europa			X		https://nap.nationalacademies.org/read/9895	National Research Council. 2000. Preventing the Forward Contamination of Europa. Washington, DC: The National Academies Press. https://doi.org/10.17226/9895.
2002	The Quarantine and Certification of Martian Samples		X			https://nap.nationalacademies.org/read/10138	National Research Council. 2002. The Quarantine and Certification of Martian Samples. Washington, DC: The National Academies Press. https://doi.org/10.17226/10138.
2002	Safe on Mars: Precursor Measurements Necessary to Support Human Operations on the Martian Surface		X			https://nap.nationalacademies.org/read/10360/chapter/1	Safe on Mars: Precursor Measurements Necessary to Support Human Operations on the Martian Surface. 2002. National Academies Press. Washington, D.C.: National Academies Press. https://nap.nationalacademies.org/catalog/10360/safe-on-mars-precursor-measurements-necessary-to-support-human-operations.
2006	Preventing the Forward Contamination of Mars		X			https://nap.nationalacademies.org/read/11381	National Research Council. 2006. Preventing the Forward Contamination of Mars. Washington, DC: The National Academies Press. https://doi.org/10.17226/11381.

Year	Title	General	Mars	Icy Worlds	Other	Text	Citation
2006	Assessment of Planetary Protection Requirements for Venus Missions: Letter Report				X	https://nap.nationalacademies.org/read/11584	National Research Council. 2006. Assessment of Planetary Protection Requirements for Venus Missions: Letter Report. Washington, DC: The National Academies Press. https://doi.org/10.17226/11584.
2009	Assessment of Planetary Protection Requirements for Mars Sample Return Missions		X			https://nap.nationalacademies.org/read/12576	National Research Council. 2009. Assessment of Planetary Protection Requirements for Mars Sample Return Missions. Washington, DC: The National Academies Press. https://doi.org/10.17226/12576.
2012	Assessment of Planetary Protection Requirements for Spacecraft Missions to Icy Solar System Bodies			X		https://nap.nationalacademies.org/read/13401	National Research Council. 2012. Assessment of Planetary Protection Requirements for Spacecraft Missions to Icy Solar System Bodies. Washington, DC: The National Academies Press. https://doi.org/10.17226/13401.
2015	Review of the MEPAG Report on Mars Special Regions		X			https://nap.nationalacademies.org/read/2181	National Academies of Sciences, Engineering, and Medicine. 2015. Review of the MEPAG Report on Mars Special Regions. Washington, DC: The National Academies Press. https://doi.org/10.17226/21816.
2017	The Goals, Rationales, and Definition of Planetary Protection: Interim Report	X				https://nap.nationalacademies.org/read/24809	National Academies of Sciences, Engineering, and Medicine. 2017. The Goals, Rationales, and Definition of Planetary Protection: Interim Report. Washington, DC: The National Academies Press. https://doi.org/10.17226/24809.
2018	Review and Assessment of Planetary Protection Policy Development Processes	X				https://nap.nationalacademies.org/read/2517	National Academies of Sciences, Engineering, and Medicine. 2018. Review and Assessment of Planetary Protection Policy Development Processes. Washington, DC: The National Academies Press. https://doi.org/10.17226/25172.
2019	Planetary Protection Classification of Sample Return Missions from the Martian Moons		X			https://nap.nationalacademies.org/read/25357	National Academies of Sciences, Engineering, and Medicine and the European Science Foundation. 2019. Planetary Protection Classification of Sample Return Missions from the Martian Moons. Washington, DC: The National Academies Press. doi: https://doi.org/10.17226/25357.
2020	Assessment of the Report of NASA's Planetary Protection Independent Review Board	X				https://nap.nationalacademies.org/read/25773	National Academies of Sciences, Engineering, and Medicine. 2020. Assessment of the Report of NASA's Planetary Protection Independent Review Board. Washington, DC: The National Academies Press. https://doi.org/10.17226/25773.
2020	Report Series: Committee on Planetary Protection: Planetary Protection for the Study of Lunar Volatiles				X	https://nap.nationalacademies.org/read/26029	National Academies of Sciences, Engineering, and Medicine. 2020. Report Series: Committee on Planetary Protection: Planetary Protection for the Study of Lunar Volatiles. Washington, DC: The National Academies Press. https://doi.org/10.17226/26029.
2021	Report Series: Committee on Planetary Protection: Evaluation of Bioburden Requirements for Mars Missions		X			https://nap.nationalacademies.org/read/26336	National Academies of Sciences, Engineering, and Medicine. 2021. Report Series: Committee on Planetary Protection: Evaluation of Bioburden Requirements for Mars Missions. Washington, DC: The National Academies Press. https://doi.org/10.17226/26336.
2023	Planetary Protection Considerations for Missions to Solar System Small Bodies: Report Series—Committee on Planetary Protection				X	https://nap.nationalacademies.org/read/26714	National Academies of Sciences, Engineering, and Medicine. 2023. Planetary Protection Considerations for Missions to Small Bodies in the Solar System: Report Series—Committee on Planetary Protection. Washington, DC: The National Academies Press. https://doi.org/10.17226/26714.

Appendix 3: PP Category II Mission Organic Inventory Template

Organic Inventory	Mission Name:

The Mission provides an itemized list of bulk organic materials [defined as all carbon-containing compounds, including payload biological materials but excluding carbides, carbonates, cyanides, and simple oxides of carbon (i.e., CO and CO_2)] presented at the same level as the materials identification and usage list (MIUL)/materials list, as used on the flight hardware, estimated actual (in kg) for organic materials present in amounts larger than 1kg; "small amounts" for organic materials present in amounts between 1kg and 0.1kg; and "traces" for identifiable organic materials present in amounts less than 0.1kg. (Add more lines as needed for each line entry.)

1. Adhesives and Potting Compounds

[e.g., RTV/Silicones (DOW, Nusil, Hysol); polyurethanes such as arathane/solithane conformal coatings; epoxies such as Scotchweld, CFRP resin]

Material Name and Usage	Actual Amount	Small Amount	Traces
		❑	❑
		❑	❑

2. Primers, Paints, and Inks

(e.g., Aeroglaze, Chemglaze, etc.)

Material Name and Usage	Actual Amount	Small Amount	Traces
		❑	❑
		❑	❑

3. Thermal Control Films

(e.g., Kapton, FEP Teflon, Betacloth)

Material Name and Usage	Actual Amount	Small Amount	Traces
		❑	❑
		❑	❑

4. Lubricants

(e.g., Braycote, molybdenum disulfide dry film)

Material Name and Usage	Actual Amount	Small Amount	Traces
		❑	❑
		❑	❑

5. Plastics and Elastomeric Materials

[e.g., circuit board with PTFE GI polyimide resin (PWB or PCB); wiring overwraps: silicones Kapton (polyimide); PEI (polyether imide); ETFE (tefzel); nylon (polyamide); PTFE (Teflon); heat shrink: Polyolefin or Polyvinylidene Fluoride types; O-rings and seals with ethyl, propyl, butyl, and Viton rubber; fluorosilicone Flectron tape (polyester); EMI shielding including Vespel (SP-1)]

Material Name and Usage	Actual Amount	Small Amount	Traces
		❑	❑
		❑	❑

6. Tapes

Adhesive tapes are principally represented by kapton (polyimide), polyester, FEP, and Teflon backings with acrylic or silicone adhesives.

Material Name and Usage	Actual Amount	Small Amount	Traces
		❑	❑
		❑	❑

7. Other

A range of other materials will need to be included, such as lacing tape and cards; thread; fibers such as Nomex; Dacron scrim cloth (polyester); DAP, Ultem PEI, PPS bulk castings; nylon (Polyamide) used in structures; fiber, Velcro, and vibe pads; propellant (MMH, UDMH); and payload biological materials (living or dead).

Material Name and Usage	Actual Amount	Small Amount	Traces
		❑	❑
		❑	❑

Appendix 4: Modeling the Probability of Contamination for NASA Missions

Overview and Scope

This appendix provides the practitioner developing the PP probability of contamination model (referred to simply as the "PP model" from this point forward) with guidance as to its roles on the project and how to formulate and develop the model throughout the project lifecycle. This section discusses purpose and applicability of the model. *Section A5.1.0 Applicable Documents* summarizes applicable governing documents, and *Section A5.2.0 Acronyms, Definitions, and Notation* defines acronyms, key terminology, and mathematical notation. *Section A5.3.0 The Role of Modeling in PP* discusses the roles modeling plays in demonstrating compliance with PP requirements and in developing the mission architecture, performing trade studies, and planning project implementation. *Section A5.4.0 Development of the PP Model* provides guidance to the practitioner on how to develop the mathematical model, while *Section A5.5.0 Examples of PP Modeling* provides several examples of previous and current models from the Jet Propulsion Laboratory (JPL), the Applied Physics Laboratory (APL), and NASA centers. A particularly useful example is the Europa Clipper Mission's PP model due to its recent development, successful implementation, and thorough documentation available in the public domain. The reader will notice elements of Europa Clipper's PP model being used to illustrate concepts and provide examples throughout this appendix. This is done while recognizing the large body of work currently being performed by other projects and organizations to assess PP contamination risk on other mission applications. When made available, information from this work will be assessed for inclusion in this appendix as part of a future revision.

Section A5.6.0 Key Behaviors of the Probability of Contamination summarizes key behaviors that previous modeling has uncovered for practitioner awareness. An addendum is provided at the end of this appendix discussing the mathematical foundations of the PP modeling *(Section A5.7.0 Addendum: Key Concepts when Formulating the Mathematical Model)*.

Purpose

The purpose of this appendix is to provide a guide to practitioners when planning, developing, and applying the mathematical model to assess the probability of contamination of NASA missions. It intends to give the practitioner awareness of the roles of different modeling techniques, including UQ and traditional PRA, and practical knowledge as to how these techniques are applied in the PP model development and how the model fits into the project lifecycle. While this appendix does not prescribe specific models or define requirements, it provides examples of models that were used for NASA missions, how requirements were interpreted and evaluated, and key behaviors of the probability of contamination identified by previous modeling that are important for any practitioner to be aware of. References to more detailed documentation or publications discussing the specific models applied are also provided.

Europa Clipper: A Common Example Used in This Appendix

The Europa Clipper Mission was implemented via a partnership between the JPL and the APL. Nine scientific instruments were selected through a NASA solicitation, with the objective of investigating Europa's habitability. Europa Clipper launched in October 2024 from Kennedy Space Center on a trajectory to Jupiter. Upon successful Jupiter orbit insertion (JOI), the spacecraft will maintain a Jupiter orbit and conduct a series of flybys of Europa (as well as a few flybys of Ganymede and Callisto), some of which are as low as 30 km in altitude. This tour phase of the Mission, where the bulk of the science data is collected, is planned to take ~4 years. At the end of the tour, the flight system will be decommissioned with an intentional impact into Ganymede.

A robust spacecraft is necessary to enable the collection of the required data and withstand the environment at Europa. Instruments are located across the spacecraft, with sensitive components requiring heavy radiation shielding, while booms and structures remain fully exposed to the local environment. The aluminum-walled avionics vault structure is of particular interest to the PP problem because it hosts electronics sensitive to both microbial reduction protocols and radiation. The vault protects hardware, but simultaneously offers a potential refuge for microorganisms. In contrast, the solar arrays are exposed to the harsh space environment; it is extremely unlikely any terrestrial organism exposed on the solar array surface at launch would survive the journey through interplanetary space and the Jovian environment to Europa.

The Europa Clipper PP model integrated elements of reliability engineering, orbital dynamics, impact physics, planetary geology, and microbiology into a single mathematical framework. This, in turn, brought together the Clipper Mission plan, bioburden, and probability of contamination to inform systems engineering decisions during Project implementation and demonstrate compliance with PP requirements.

Applicability

This appendix applies to all models developed to assess the probability of contamination requirements set forth by NASA. Specifically, this includes:

- Both forward and backward contamination cases
- Both remote space systems (e.g., orbiters, flyby vehicles) and non-remote (in situ) systems (e.g., landers, probes)

In its current form, this appendix does not include guidance for:

- Bioburden threshold requirements for Mars Missions
- PP-related risk assessments being performed for crewed missions, including Artemis and Mars-to-Moon Missions

This appendix is written for those personnel developing probabilistic models to assess PP-related contamination risk. This appendix does not have authority to levy new or interpret current requirements on missions. Instead, it provides guidance as to how the requirements can be addressed via models, assuming that such authorities exist to set forth and adjudicate compliance with these requirements.

Applicable Documents

General

The documents listed in this appendix are applicable to the guidance in this Handbook. The latest issuances of cited documents applies unless specific versions are designated.

The applicable documents are accessible via the NASA Technical Standards and Technical Assistance Resource Tool at **http://standards.nasa.gov** or may be obtained directly from the Standards Developing Organizations or from other document distributors.

Government Documents

NASA/SP-2011-3421	Probabilistic Risk Assessment Procedures Guide for NASA Managers and Practitioners
NPR 7120.5F	NASA Space Flight Program and Project Management Requirements w/Change 1
NPR 8715.24	Planetary Protection Provisions for Robotic Extraterrestrial Missions
NASA-STD-8719.27	Implementing Planetary Protection Requirements for Space Flight
NPR 7150.2	NASA Software Engineering Requirements

Acronyms, Definitions, and Notation

Acronyms and Abbreviations

APL	Applied Physics Laboratory
BAT	Best Available Technology
CBE	Current Best Estimate
CDR	Critical Design Review
DDL	Deorbit, Descent, and Landing
FFRDC	Federally Funded Research and Development Center
JIMO	Jupiter Icy Moons Orbiter
JOI	Jupiter Orbit Insertion
JPL	Jet Propulsion Laboratory
NASA	National Aeronautics and Space Administration

NPR	NASA Procedural Requirements
OPP	Office of Planetary Protection
OSMA	Office of Safety and Mission Assurance
PDR	Preliminary Design Review
pH	Negative log of the hydrogen ion concentration
PP	Planetary Protection
PPEL	Planetary Protection Equipment List
PRA	Probabilistic Risk Assessment
PSE	Project Systems Engineering
RQ	Requirement
SIR	System Integration Review
UQ	Uncertainty Qualification
V&V	Verification and Validation

Definitions

Backward Contamination Harmful biological contamination of the Earth-Moon system by potential extraterrestrial life and bioactive molecules in returned samples from habitable worlds.

Exchangeability The joint probability that a set of random variables is the same no matter how the values taken by the random variables are swapped around (exchanged); that is, $p(X_1 = x_1, X_2 = x_2, \ldots, X_n = x_n) = p(X_1 = x_{\sigma(1)}, X_2 = x_{\sigma(2)}, \ldots, X_n = x_{\sigma(n)})$, where σ is any permutation of the indices $\{1, \ldots, n\}$.

Forward Contamination Harmful contamination of other worlds by terrestrial organisms, organic materials, and volatiles carried or released by spacecraft.

Independence One event does not provide any further information to affect the probability of another event. Mathematically, two events A and B are independent if and only if $p(A \mid B) = p(A)$.

Mutual Exclusivity Two events share no outcomes in common. Mathematically, two events A and B are mutually exclusive if and only if $p(A \cap B) = 0$.

Resurfacing The geologically facilitated start of surface modification, potentially initiating transfer to the subsurface, which may or may not bring a microorganism that was introduced by robotic exploration into contact with liquid water.

Mathematical Notation and Theorems

Pc The probability of contamination as estimated by the PP model.

A, B Events; sets of possible outcomes that have a well-defined probability.

p(A | B) The conditional probability function of an event A given another event B.

p(A) The probability function of the event A conditioned on the entire set of possible outcomes when this set of outcomes is understood.

X, Y Random variables or random vectors (functions from the set of possible outcomes to real numbers).

p(X | Y) The conditional probability function of a random variable (or random vector) X given another random variable (vector) Y. When X is discrete, $p(X \mid Y)$ is understood to be a probability mass function; when X is continuous, $p(X \mid Y)$ is understood to be a probability density function.

p(X) The marginal probability function of a random variable (or random vector) X.

$p(x \mid y)$	The conditional probability function of a random variable (or random vector) X taking the value x given another random variable (vector) Y takes the value y. When X is discrete, $p(x \mid y)$ is understood to be a probability mass function; when X is continuous, $p(x \mid y)$ is understood to be a probability density function.
$p(x)$	The marginal probability function of a random variable (or random vector) X taking the value x.
Bayes' Theorem	The mathematical theorem showing how to invert the conditional probability of one event on another; for any two events A and B where $p(B)>0$, $p(A \mid B) = \frac{p(B \mid A) \times p(A)}{p(B)}$.
de Finetti's Theorem	The mathematical theorem that asserts the existence of an event that, when conditioned on, allows a set of exchangeable random variables to be treated as independent of one another; for any sequence of exchangeable random variables $X_1, \ldots, X_n$, there exists a conditioning event E such that $p(X_1, \ldots, X_n \mid E) = \prod_{i=1}^{n} p(X_i \mid E)$.

The Role of Modeling in PP

The role of the PP model for a project depends on the maturity of the project and the project priorities given available resources. The specific roles of the PP modeling on flight projects, concept studies, and proposals may include:

- Development of a modeling approach that is capable of demonstrating compliance with PP requirements in the future
- Informing trades as to the probability of contamination of various architectures
- Identifying drivers of PP contamination risk
- Informing plans having to do with bioburden reduction measures (e.g., bakeouts, cleaning, and other pre-launch bioburden reduction measures)
- Being part of (but not replacing) a larger PRA
- Demonstrating compliance with PP requirements

The modeling can provide more information to a project than just demonstrating compliance with PP requirements. Once the model is sufficiently developed, it will play a key role in PP-related trades and architecture decisions during early formulation of the mission concept. This was demonstrated for the Europa Clipper Mission and Europa Lander concept; see *Section A5.5.0 Examples of PP Modeling* and McCoy 2021[163] for specific examples and further discussion.

Development of the PP Model

The PP model uses a mathematical representation of a system to assess quantities or events of interest. While physics-based or other phenomenological models are incorporated to accurately represent these systems, the presence of uncertainty requires these models to be wrapped into a larger probabilistic architecture. These models can take various forms depending on the risk tolerance of applicable stakeholders (many times reflected in the mission risk classification or PP categorization), requirements, resources available to the project, and current state-of-knowledge of the system. Figure 44 summarizes these different types of analysis, discussed further below:

- **Deterministic analysis:** This is sometimes used when no probabilistic requirement exists or it is not important to the project or other stakeholders to quantify uncertainty. A purely deterministic analysis cannot provide a risk assessment on its own (hence the lack of an arrow from this box to the PP model in Figure 44) but can provide initial insights or inputs to be considered by a future risk assessment.
- **Bounding/sensitivity analysis:** Sometimes a relatively quick bounding analysis looking at worst cases is adequate to rule out a risk or determine if further analysis is needed in order to understand the level of risk of the system. Other times, a phenomenon may not be understood well enough to describe it in probabilistic terms. In such cases, sensitivity analysis can be performed on the model parameters to bound the effect of the phenomenon on the risk.

[163] McCoy K, DiNicola M, Everline C, Burgoyne H, Reinholtz K, Clement B, *Europa Clipper planetary protection probabilistic risk assessment summary*, Planetary and Space Science, 2021

- ***Example bounding/sensitivity analysis:*** The rate at which geological activity replaces surface features on Europa (resurfacing), which brings material on the surface into contact with the subsurface (potentially liquid water), is constrained to be between one event every 200 Myrs *and* one event every 20 Myrs. While this is a key parameter in the modeling of the probability of contamination of Europa, a probability distribution describing the relative likelihood of this resurfacing rate is unknown. In this case, the contamination risk of spacecraft hardware delivered to Europa can at most be bounded through sensitivity analysis of values over this interval, and the risk described by "best case" and "worst case" probabilities depending on the results of the sensitivity analysis.

- **Uncertainty Quantification (UQ) analysis:** If the phenomenon associated with the risk assessment can be described probabilistically, then a UQ analysis bringing together physics-based or other phenomenological models (many times from a wide variety of disciplines) within a probabilistic architecture can be developed. These models can provide a rigorous mathematical approach to quantifying risk to support making systems engineering decisions under uncertainty. Models must undergo verification and validation (V&V) and be informed by credible data.
  - ***Example UQ analysis:*** The Europa Clipper PP model borrowed from a large suite of physics-based and phenomenological models to describe spacecraft reliability, possible impact trajectories, breakup of the spacecraft at impact, geological activity, and microorganism survival to bound the probability of contamination. This was done within a probabilistic framework. Models underwent significant V&V and were reviewed extensively by stakeholders and a panel of independent experts for validity and credibility as part of a dedicated three-day workshop.
- **Probabilistic Risk Assessment (PRA):** A formal process that plays a fundamental role in the risk assessment process for NASA flight projects, particularly in the area of reliability engineering. It provides a systematic approach to identifying and quantifying risk for complex systems using validated tools and methods. In general, risk in PRA is defined as a triplet composed of a scenario, likelihood, and consequence.[164] The risk triplet involves three questions:
  - What can go wrong?
  - How likely is it?
  - What are the consequences?

  Development of a PRA involves first defining the end states of concern. The scenarios that lead to these end states are then logically modeled and quantified to calculate the probability of the undesired end state. PRA is intended to be used to address high-stakes scenarios for a system, such as those that could lead to harm to or loss of human life, loss of mission, or failure to satisfy Level 1 requirements.
  - Example PRA: See the detailed handbook for NASA PRA[165] for several examples. The PRA can form part of a larger risk analysis as well, although the scope and benefits of PRA can be affected by other models being used outside the PRA. For instance, the resurfacing model discussed in the above example made a detailed PRA assessment of spacecraft reliability unnecessary (at least for PP purposes).

Note that these different forms of analysis are not necessarily mutually exclusive. For example, PRA can be used as a tool within a larger UQ analysis. Many of the techniques of PRA are employed in UQ and vice versa, especially since both have strong foundations in probability theory and Bayesian statistics. Both PRA and UQ use sensitivity analysis to validate their models and bound risk in certain scenarios.

[164]Risk can be defined differently outside of the PRA arena; for instance, reliability assessments may define risk as the maximum loss to the mission. In some statistical disciplines, risk is defined as the mean-squared error. In decision theory or the insurance industry, risk might be defined in terms of an expected loss or premium.
[165]NASA/SP-2011-3421, Second Edition, Probabilistic Risk Assessment Procedures Guide for NASA Managers and Practitioners, December 2011

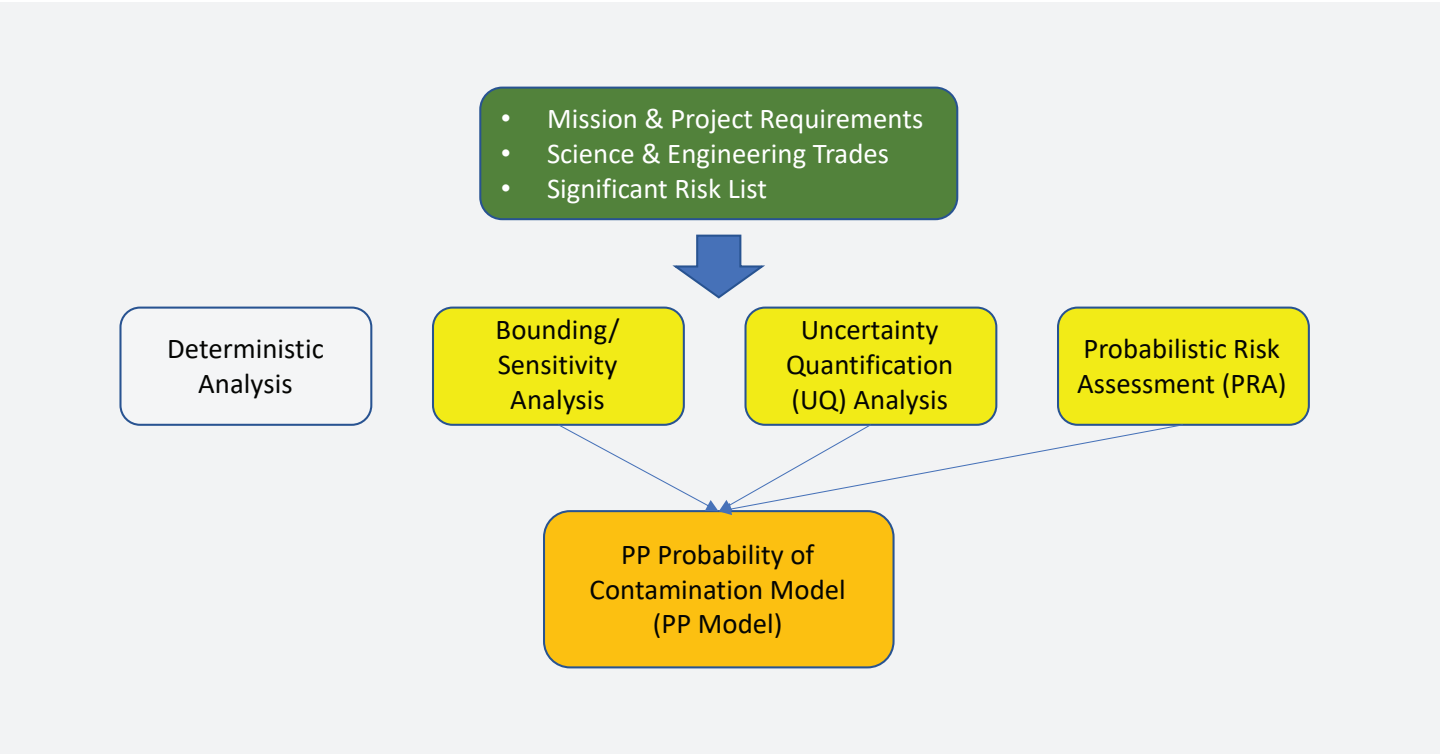

Figure 35: *The Probability of Contamination Model*
Note: The model utilizes appropriate modeling approaches to assess contamination risk depending on the risk tolerance of applicable stakeholders (many times reflected in the mission classification or categorization), NASA requirements, resources available to the project, and current state-of-knowledge of the system.

As described in *Section 1.1.0 Background*, the main objectives of PP are to:

1. Carefully control forward contamination of other worlds by terrestrial organisms and organic materials carried by spacecraft in order to guarantee the integrity of the search and study of extraterrestrial life, if it exists.
2. Rigorously preclude backward contamination of Earth by extraterrestrial life or bioactive molecules in returned samples from habitable worlds in order to prevent potentially harmful consequences for humans and the Earth's biosphere.

Development of the mathematical model involves first defining a criterion to be evaluated, typically a criterion that defines the success of the system or mission being analyzed. This criterion is then used to formulate a mathematical model that calculates the probability of satisfying or not satisfying the success criteria. The mathematical formulation is critical, as it provides the roadmap for how the model is calculated and how all further modeling work is integrated; hence, it is a key management tool as well. Alongside this, it is often helpful to develop an event tree;[166] that is, a causal sequence of pivotal events having binary outcomes that culminate in the success or failure of the system. This also serves as a very useful communication device when explaining the model logic to project management and other stakeholders. Individual mathematical models are then developed for each pivotal event and used to assess the probability of each outcome. These models are constructed in a way that integrates back into the mathematical formulation developed to access the success criteria. All models are verified and validated, sensitivity analysis being a key aspect of this to understand the behaviors of the models and test the model's validity.

This general approach is applied to develop the mathematical model for PP when assessing the probability of contamination (see Figure 36); once a plan is put in place, a success criteria is articulated by defining a contamination event and what it means for a mission to successfully avoid contamination. With these criteria put in place, a mathematical formulation governing the probability of contamination can then be developed along with an accompanying event tree to describe the end-to-end sequence of events that lead to contamination events. Examples of event trees for PP and associated pivotal events are

[166]Equivalently, an Event Sequence Diagram can be used to convey the same information in a decision tree format, which is sometimes easier to communicate to reviewers, stakeholders, and decision makers.

given below. Lower-tier submodels are then constructed to evaluate each element of the event tree. V&V of all submodels and the overarching model itself is performed. Finally, an assurance case may be developed that communicates the model results to stakeholders and decision-makers. Each of these parts of the modeling process are discussed in further detail below.

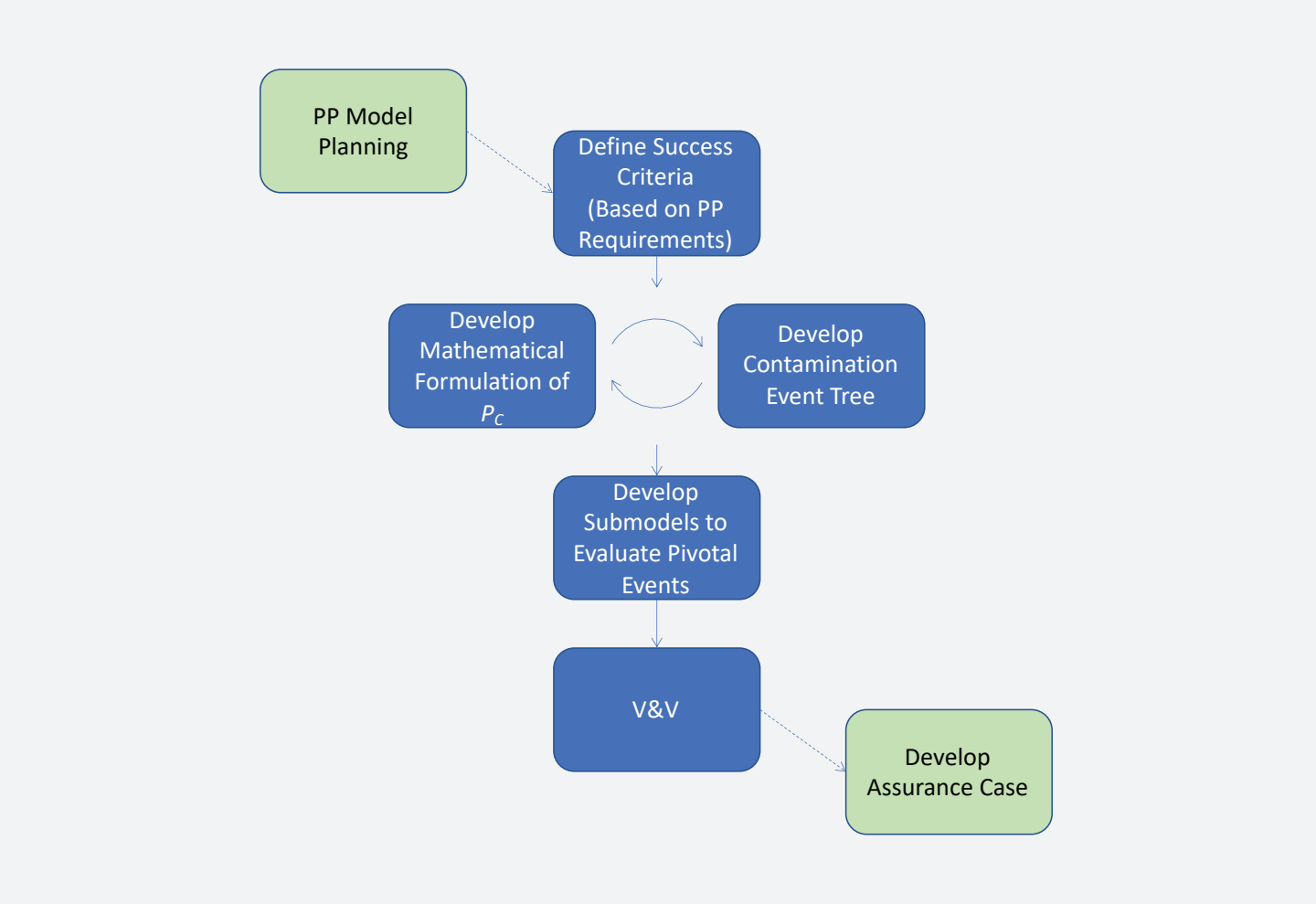

Figure 36: *Model Development Process for Evaluating the Probability of Contamination*

Roles and Responsibilities

Development of the PP model cannot be performed by PP practitioners in a silo. It is an inherently multidisciplinary effort that, depending on the mission, may require mathematicians, statisticians, PP engineers, reliability engineers, mission designers, experts in fluid dynamics, planetary scientists, microbiologists, and possibly others. The PP modeling team must be involved in the development of any submodels developed by these SMEs in order to ensure they can be integrated within the probabilistic architecture. For Europa Clipper, the PP modeling team was in close contact with the PP Lead and part of the larger Project Systems Engineering (PSE) team to interpret requirements, define the model and objectives, inform systems engineering decisions affecting the Project, and coordinate with NASA OPP for reviews of the PP model development and results. If a PRA is used by a mission, the project works closely with the mission assurance technical authority to develop the PRA and integrate it into the larger PP model.

Sizing the Modeling Effort

One of the first conversations the PP modeling team lead has with the project is focused on the amount of resources it will take to develop the PP model and to what depth. While this amount is fundamentally limited by available project resources

(funds, time, and personnel), a good place to start is to identify stakeholders and decision-makers and then elicit from them topics, questions, or objectives they would like addressed by the model. Obviously, any project Level 1 PP requirements will need to be addressed. This is a threshold modeling effort that must be performed. Beyond this, however, there may be hardware trades, implementation plans, architecture decisions, and other risks that are sensitive to the probability of contamination. The model can be further enhanced beyond the threshold effort to address these engineering decisions as well.

While topics and objectives scope the ultimate goal of the modeling effort, the phases in the project lifecycle will play a significant role in determining how far to go with the modeling to address these objectives. For instance, early in formulation, it is not necessary to demonstrate compliance with PP requirements, but it is important to develop the model enough to have a credible and valid approach to demonstrate compliance in the future.

- ***Example (Europa Lander):*** Europa Lander developed its PP model during an advanced development phase in pre-Phase A. During this time, the model was developed to address certain key trades and architecture decisions having to do with the inclusion and sizing of an in-flight terminal sterilization system and biobarrier. The objective was not to demonstrate compliance with the PP requirement at this early phase, but to demonstrate a credible path to compliance. A successful peer review was held with NASA OPP and SMEs to ensure the model could credibly address these objectives.

In practice, objectives of the modeling will mature with the project and some flexibility needs to be allowed. Typically, a "peel-back-the-onion" approach to investigating risk is practical and allows the model to grow as necessary, provided there is data. This expands the model and risk analysis one layer at a time, allowing management and stakeholders to evaluate if more detailed modeling or additional resources or data are needed to appropriately address the questions from the project, while staying within available resources. If additional resources are determined to be necessary to address project questions or objectives, project management should be informed and resources prioritized accordingly.

- ***Example (Europa Clipper):*** The Europa Clipper PP model demonstrated that the contamination probability up to the moment of impact was not sufficient to demonstrate compliance with the PP requirement. Therefore, the Project decided to invest additional resources to assess impact heating and survival as the next most credible path to compliance. This also proved insufficient. Finally, as the next most promising step, the Project decided to invest in a geological resurfacing model and utilize a "period of biological exploration" constraint. Compliance was demonstrated after investigation of this last layer of model expansion.

Planning for the PP Model Development

As previously discussed, the role of the PP model on a project depends on the maturity and needs of the project. Figure 37 shows how a notional development schedule for the PP probability of contamination model may look based on previous modeling efforts. While this schedule does not impose additional requirements, it is recommended that a project use this information to best meet requirements that already exist (such as compliance with PP contamination requirements). The depth and rigor of the model will be determined at least in part by the preliminary PP mission categorization and requirements as assessed in pre-Phase A, and the final categorization and requirements in Phase A. This will help the project and PP modeling team scope the PP model, determine what new modeling is necessary and what can be reused from previous work, and develop these new models as needed. Importantly, a PRA may be used; if so, a PRA plan will need to be developed as part of the overall development of the PP model.

During Phase A, the PP probability of contamination model should be developed enough to inform top-level trades and architecture decisions being made by the project. By the Preliminary Design Review (PDR) timeframe, the PP model should be fully reviewed by V&V and demonstrate compliance with the PP requirement. Consistent with past assessments of the probability of contamination, it is recommended that a peer review or workshop (definitely one, possibly both) of the PP model be held by this time with NASA OPP and a panel of independent SMEs from each discipline utilized in the modeling. Quarterly meetings with NASA OPP were helpful in the past to keep NASA OPP up to date on the model development and any results. An assurance case approach may be recommended to decision-makers by the end of Phase B as well. That said, the details of the composition of an assurance case are still being developed on a case-by-case basis within the PP discipline.

Throughout Phases A and B, and up to Critical Design Review (CDR), the PP model should be used to inform the PP Implementation Plan and parts selection. Advanced developments in pre-Phase A may also develop the PP model to inform decisions at this early phase of the lifecycle. The contamination risk of long lead parts may need to be addressed as early as Phase A. Prior to Phase B, the PP model should be developed enough to determine a path to compliance and demonstrate compliance of the mission with PP requirements by PDR. After PDR, the PP model is maintained and used to demonstrate continued compliance with the PP requirements as the mission moves to launch. The PP model is also used throughout mission operations to assess the PP contamination risk associated with critical events (e.g., maneuvers that may have a high probability of contaminating the body of concern or that could expose the Earth-Moon system to contaminants from other planetary bodies); mission design re-plans; end-of-mission disposal of the spacecraft; extended mission designs; and, for restricted sample return missions, safe delivery, containment, and curation of extraterrestrial materials.

PP Model Activity	Notional PP Model Development Schedule[167,168]						
	Pre-Phase A	Phase A	Phase B	Phase C, thru CDR	Phase C/D, post CDR	Phase E	Phase F/Ext Mission
PP Model Planning, Develop Success Criteria	Prelim PP categorization, RQs	Final PP categorization, RQs					
Develop PP Model		Peer Review	V&V complete, Workshop				
Inform Trades and Architecture Decisions	Advanced developments						
Inform Parts Selection, PP Implementation Plan		Long lead parts	Baseline PP Imp Plan				
Demonstrate Compliance w/PP RQs		Path to compliance	Demonstrate compliance, Assurance Case			Critical events	Spacecraft disposal, sample curation

Figure 37: *Notional PP Model Development Schedule*

Definition of the Success Criteria

The success criteria for the PP model is motivated largely by the PP requirements. This success criterion is defined by considering three elements:

1. The quantity or event of interest.
2. An acceptable value for the quantity of interest not to exceed probability of the event of interest.
3. Acceptable events that may be considered by the model, and acceptable methods and data that may be used to calibrate the model. There may be higher-level mission success criteria that the PP model fits into or system success criteria that are related to the PP model that need to be considered as well.

[167]Lifecycle based on NPR 7120.5F [NASA Procedural Requirements (NPR) 7120.5F, NASA Space Flight Program and Project Management Requirements w/Change 1, Associate Administrator, effective from August 3, 2021 – August 3, 2026] and nominal document schedule in NPR 8715.24 (Table 3-2) [NASA Procedural Requirements (NPR) 8715.24, Planetary Protection Provisions for Robotic Extraterrestrial Missions, Office of Safety and Mission Assurance, effective from September 24, 2021 – September 24, 2026].
[168]The actual schedule of activities for the modeling is negotiable.

For forward contamination, the PP requirement and associated documents provide definitions of the terms used in this criterion and provide the conditions by which the risk of contamination is acceptable.[169] Backwards contamination was not as clearly defined at the time this appendix was written, using "target" thresholds for risk but with no requirement(s) described.[170] An acceptable risk threshold, acceptable techniques, methods and data to demonstrate the risk is acceptable, and a decision authority to adjudicate these matters is critical to establishing credibility of the model results and decisions as to whether the risk is acceptable or not. Further discussion of these matters is outside the scope of this appendix.

To illustrate, we look at forward contamination of ocean worlds. As previously stated, the PP requirement provides much of the information needed to consider (1)-(3) above. Paraphrasing[171] from NASA-STD-8719.27,[172] the PP probability of contamination requirement states:

Missions to sensitive icy worlds (e.g., Europa, Enceladus) must demonstrate a less than 1×10^{-4} probability that a biological inoculation event occurs to a habitable environment on these worlds within the period of biological exploration (i.e., by the year 3000), where:

- A biological inoculation event is the introduction of one or more viable organism(s) to a solar system body capable of providing nutrients and environmental growth conditions such that the organism can replicate.
- A habitable environment is an environment where physico-chemical limits (availability of water and biomolecule building blocks, presence of an energy source/redox gradient, and permissive temperature and pH ranges) permit replication of carbon-based living organisms.

The definition of a habitable environment above has direct implication to the scope of the PP model. Project management, scientists, and PP representatives should be engaged to define what this means for the planetary bodies potentially affected by the mission, and what burden of proof might be necessary for demonstrating compliance with PP requirements. For instance, in the case of an ocean world, the following environments may warrant consideration:

- Subsurface ocean and thermal vents
- Interstitial liquid water (e.g., conduits, diapirs)
- Surface vents (e.g., Enceladus, maybe Europa)
- Other surfaces of the ice shell
- Plume material (e.g., Enceladus, maybe Europa)
- Liquid water made by interactions between spacecraft and the target body's biosphere (e.g., Enceladus)
- Other bodies that could come into contact with the target body or spacecraft (e.g., Enceladus plume material contacting Saturn's E-ring or a Europa spacecraft impacting Ganymede or Callisto)

Investigating the contamination risk of some of these environments may require new analyses or have a risk that is simply not quantifiable at the time. If the contamination risk cannot be quantified (due to lack of knowledge or not being a cost-effective use of available resources), the risk could be discussed as part of an assurance case (see the following page).

Continuing with NASA-STD-8719.27,[173] contamination avoidance can be demonstrated through data and analyses of:

- Bioburden at launch
- Cruise survival for contaminating organisms
- Organism survival in the environment adjacent to the target
- Probability of encountering/landing on the target
- Probability of surviving landing/impact on the target
- Mechanisms and timescales of transport to the subsurface
- Organism survival and proliferation before, during, and after subsurface transfer

[169] While an acceptable risk threshold is articulated in NASA-STD-8719.27 [NASA Technical Standard (NASA-STD) 8719.27, Implementing Planetary Protection Requirements for Space Flight, Office of Safety and Mission Assurance, approved August 30, 2022], no justification for why this value was chosen is provided.

[170] **https://downloads.regulations.gov/NASA-2022-0002-0176/content.pdf**

[171] The actual statement from NASA-STD-8719.27 (see footnote 168) for Category III missions is "missions conducting a flyby or gravity assist of Europa, Enceladus, or other sensitive icy worlds to be determined shall demonstrate contamination avoidance at a probability of occurrence less than 1.0 x 10-4 for a biological inoculation event into a potentially habitable aqueous environment (e.g., liquid water body, brine) for 1,000 years.

[172] See footnote 168.

[173] See footnote 168.

In summary, the success criterion is defined as follows: the event of interest is a contamination event, the not-to-exceed probability of this event is 1×10^{-4}, and the data and analyses above provide acceptable and complete considerations when assessing this risk of a contamination event in the PP model. The mathematical formulation and other model developments in the next section will rigorously capture all quantifiable aspects of the PP model as defined by the success criteria. The acceptability of the methods, techniques, and data used in the modeling is subject to the evaluation of an appropriate approval authority. For NASA missions in the past, this has taken the form of a review or workshop that includes all stakeholders and a committee of independent SMEs.

Development of the Mathematical Formulation of P_C

This section discusses some of the critical notions and principles underlying most mathematical model developments, including formulations of the probability of contamination, denoted by P_c. A top-level derivation of the Europa Clipper's PP model mathematical formulation is provided to demonstrate the application of these notions. Important considerations for PP model developments for different types of missions are also briefly discussed.

Notions of conditional probability, independence, and mutual exclusivity are fundamental to probability theory and used extensively in the development of the mathematical formulation of the PP model. Mutual exclusivity is a mathematical concept that simplifies modeling by partitioning events so that the probability of the union of all events is the sum of the probabilities of the individual events. Conditional probability allows us to design our model to capture all relevant dependencies that may exist, and many times are associated with non-linear model behavior (see *Section A5.4.0 Development of the PP Model* for an example). Independence is another key notion that can be leveraged to simplify the modeling, especially when appropriate conditions can be quantified for when one event does not provide any further information to affect the probability of another event. Conditional probability combines with the notion of independence via de Finetti's theorem to bridge the gap between physics-based or phenomenological models and probability theory. Bayes' theorem then allows the probabilistic model to be informed by data in a mathematically rigorous way for statistical analysis. See *A5.7.0 Addendum: Key Concepts when Formulating the Mathematical Model* for an in-depth mathematical discussion of these concepts.

Modeling of Backward vs. Forward Contamination

An important difference between the modeling of backward contamination and forward contamination is how uncertainty is captured in biological models. Terrestrial life has some basis in empirical investigations, and experiments can be performed to develop survival and bioburden models. This is in contrast to life or other entities of contamination concern originating on other worlds, where our knowledge is speculative and typically shaped by our understanding of terrestrial life. If modeling is performed, uncertainty needs to be quantified credibly with sound probabilistic and statistical methods. Modeling should also be upfront with its limitations, and PP modeling is no exception.

Modeling of Remote Sensing vs. In Situ Missions

Modeling of remote sensing (e.g., orbiters, flyby spacecraft) and in situ missions (e.g., landers, probes) differ in that remote sensing missions do not nominally contact the PP body of concern, whereas in situ missions do. For in situ missions, the modeling bifurcates to consider contamination events from the nominal mission and those from off-nominal scenarios. Contamination is only possible for remote sensing missions if certain off-nominal scenarios occur.

Application To Europa Clipper

The mathematical formulation from the Europa Clipper PP model provides a useful illustration. As found in McCoy 2021,[174] the mathematical formulation of the probability of contamination is

$$P_C = \sum_{n=1}^{N} p(C_{n,t})$$
$$= \sum_{n=1}^{N} p(I_n) \, x \, p(R_t \mid I_n) \, x \, p(S \mid R_t \cap I_n),$$

where

- $C_{n,t}$ is the event that the spacecraft completes its first n-1 maneuvers but contamination occurs due to a failure to complete maneuver *n*
- I_n is the event that the spacecraft successfully completes the first n-1 maneuvers, a failure occurs after maneuver n-1 that causes the spacecraft to not complete maneuver *n*, and the spacecraft cannot be recovered in time to avoid impact with Europa
- R_t is the event that a microorganism, originally on board the spacecraft, resides on an area of Europa that resurfaces within the period of biological exploration defined by *t*; and
- *S* is the event that one or more microorganisms launched with the spacecraft survive to come in contact with the Europan subsurface.

Here, $C_{n,t}$ is defined as the conjunction of events I_n, R_t and *S*; i.e., $C_{n,t} = I_n \cap R_t \cap S$. Each of the events $C_{(n,t)}$ are mutually exclusive and so the probability that contamination occurs from any segment of the Europa tour is the sum of the probabilities that contamination occurs due to a failure to complete maneuver *n*; i.e., $p(C_{(n,t)})$. This gives the first equality above. The second equality follows from the definition of conditional probability, where events are conditioned one by one in their "causal" or "information-oriented" order. First, failure and impact of the spacecraft occurs; this leads to certain impact characteristics (e.g., impact speed, obliquity, location) that inform how debris and microorganisms would disperse over the surface of Europa. Geological processes would then bring materials, potentially microorganisms, into the subsurface of Europa and to liquid water. The impact characteristics, debris dispersion, current knowledge of the Europan radiation environment, and microorganism mortality give all the information needed to calculate the probability that one or more microorganisms survive this journey. This information, together with mortality functions, essentially provides a model of microorganism survival in the context of a contamination event by Clipper. Thus, de Finetti's theorem is invoked,[175] and microorganisms are treated as (conditionally) independent of one another when calculating $p(S \mid R_t \cap I_n)$. For further discussion, see McCoy 2021[176] and DiNicola 2022.[177] This independence of individual microorganism survival, once conditioned on trajectory, resurfacing events, spacecraft location, and organism type, is fundamental to the key model behavior discussed in *A5.7.0 Addendum: Key Concepts when Formulating the Mathematical Model*.

Development of the Contamination Event Tree

An event tree is a graphical display of the key events that lead to a contamination event and is a valuable tool when communicating to stakeholders and decision-makers. It is also a useful organizational tool when formulating the mathematical model. Depending on what events succeed or fail in the event tree, a well-defined outcome is articulated. The construction of the event tree is done such that all outcomes are mutually exclusive, and together, exhaust all possibilities. Of course, the outcomes must also include the event or quantity of interest in a way that can be evaluated by the success criteria (*Section A5.4.0 Development of the PP Model*). This allows outcomes to be clearly delineated and probabilities associated with each scenario or outcome to be easily summarized when reporting results; in particular, those for the event/quantity of interest.

Application To Europa Clipper

Figure 38 shows the Europa Clipper Contamination Event Tree, with the mathematical formulation at the top of the figure to show how it relates to the event tree. This figure illustrates the events necessary to culminate in a contamination event. The

[174] See footnote 162.

[175] Microorganism survival of a given group and in a given bioregion is treated as exchangeable: consider the joint probability distribution of a set of Bernoulli random variables, each representing an individual microorganism's survival by a "1"; death by a "0".

[176] See footnote 162.

[177] DiNicola M, Howell S, McCoy K, Burgoyne H, Hasnain Z, Reinholtz K, Fleischer S, *Resurfacing: An Approach to Planetary Protection for Geologically Active Ocean Worlds*, The Planetary Science Journal, 2022

current scope of the Europa Clipper model, from a temporal perspective, begins with a successful launch and a bifurcation at JOI (leftmost node of the Event Tree), depending on whether or not JOI is achieved. If JOI is not achieved, contamination via Clipper cannot occur (denoted by a star shape in the node).

Within the time period between successful JOI and a potential inadvertent impact (denoted by P_{Impact} in the Event Tree), recoverable and non-recoverable maneuverability failures are assessed. A decision was made when planning the PRA to assess and model recoverable failures because of the nature of the Europa flybys and the close proximity at which they come to the body. This added complexity to the problem because recovery time relative to impact time also needed to be understood and modeled. If Clipper fails to perform a planned maneuver, there is some probability (resulting from uncertainty in the exact dynamical properties of Clipper at the time the maneuver failed) that Clipper will follow a trajectory that impacts Europa. If Clipper is on such a trajectory and the failure is non-recoverable, impact will occur. If Clipper is on an impact trajectory but the failure is recoverable and recovery occurs at least a certain amount of time prior to impact, then impact can be avoided. Assuming either of the two scenarios can occur at any point in the mission prior to proper disposal, the model must calculate impact probability for each trajectory resulting from each of the ~200 maneuvers planned in the nominal mission, in the event spacecraft recovery does not occur in time to prevent impact. It is important to note that impact of Europa cannot occur unless Clipper fails to properly perform a maneuver.

In the case that Clipper inadvertently impacts Europa, cratering will occur and debris will scatter across the surface of Europa. Geological activity often referred to as “resurfacing” may bring microorganisms delivered to Europa by Clipper into contact with the Europan subsurface. This event can be seen in the middle orange segment of the event tree. Correspondingly, in the event an impact of Europa occurs and a resurfacing event brings a microorganism delivered by Clipper into contact with the Europan subsurface, the probability anything survives to contaminate Europa must be considered. If all microorganisms have died prior to transmission to the Europa subsurface, the probability of contamination is zero. It is also important to note that it is assumed that a viable microorganism aboard the Clipper flight system cannot contaminate Europa if the flight system does not impact Europa. These survival-related elements of the event tree are shown boxed in green in Figure 38.

The lethality factors that determine survivability of microorganisms from launch up to an inadvertent impact and until a resurfacing event occurs are: ionizing radiation, low temperature, low water activity, exposure to a space vacuum/environment, and impact heating. Lethality factors once sufficiently below the surface are not well understood. Additional research and modeling are needed to assess this as well as modes of transfer through the ice shell to liquid water. Once this data is available, the probability of contamination calculation can be expanded to include such variables (see rightmost segment of the event tree). However, expansion of the model for Europa Clipper ceased when the PP contamination avoidance requirement was met, and so the model was not expanded to assess transfer and survival events beyond resurfacing (e.g., P_{Prolif} in the event tree).

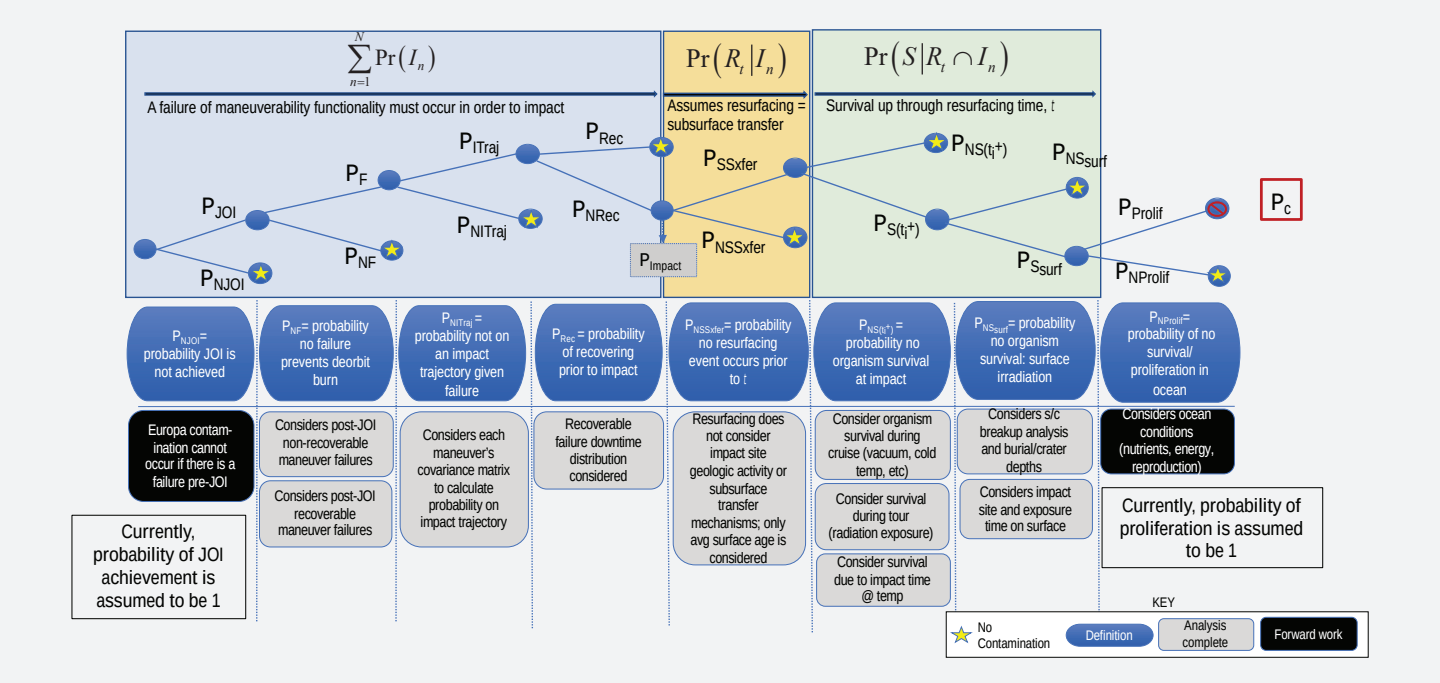

Figure 38: *Europa Clipper Contamination Event Tree*

Development of Submodels To Evaluate Pivotal Events: Europa Lander

When constructing event trees, each pivotal event will have an associated model to determine its success or failure. These models, or submodels, might be reliability models if the pivotal event involves hardware functionality; trajectory models if the pivotal event involves an impact of the spacecraft with another body; geological models if the event involves interactions of the planetary body with potential contaminants; or biological models if the event considers microorganism survival. Moreover, these submodels may be dependent on one another given dependencies in the event tree. Figure 39 shows the network of submodels and analyses underlying the preliminary Europa Lander PP model, developed as part of a JPL study. As can be seen, the PP model is composed of three main submodels: Impact/Contact, Resurfacing, and Biological. The PPEL is a key input to the Biological submodel, provided by the PP engineer. The blue, red, and green chart elements denote other analyses and submodels required for the PP model. Directional arrows show where key dependencies between submodels exist and how one model feeds another. This color-coding shows the heritage of the Europa Lander PP model with the PP model developed for the previously discussed Europa Clipper Mission, which was useful for planning the resources for this successor model and communicating the scope of the work effort with management.

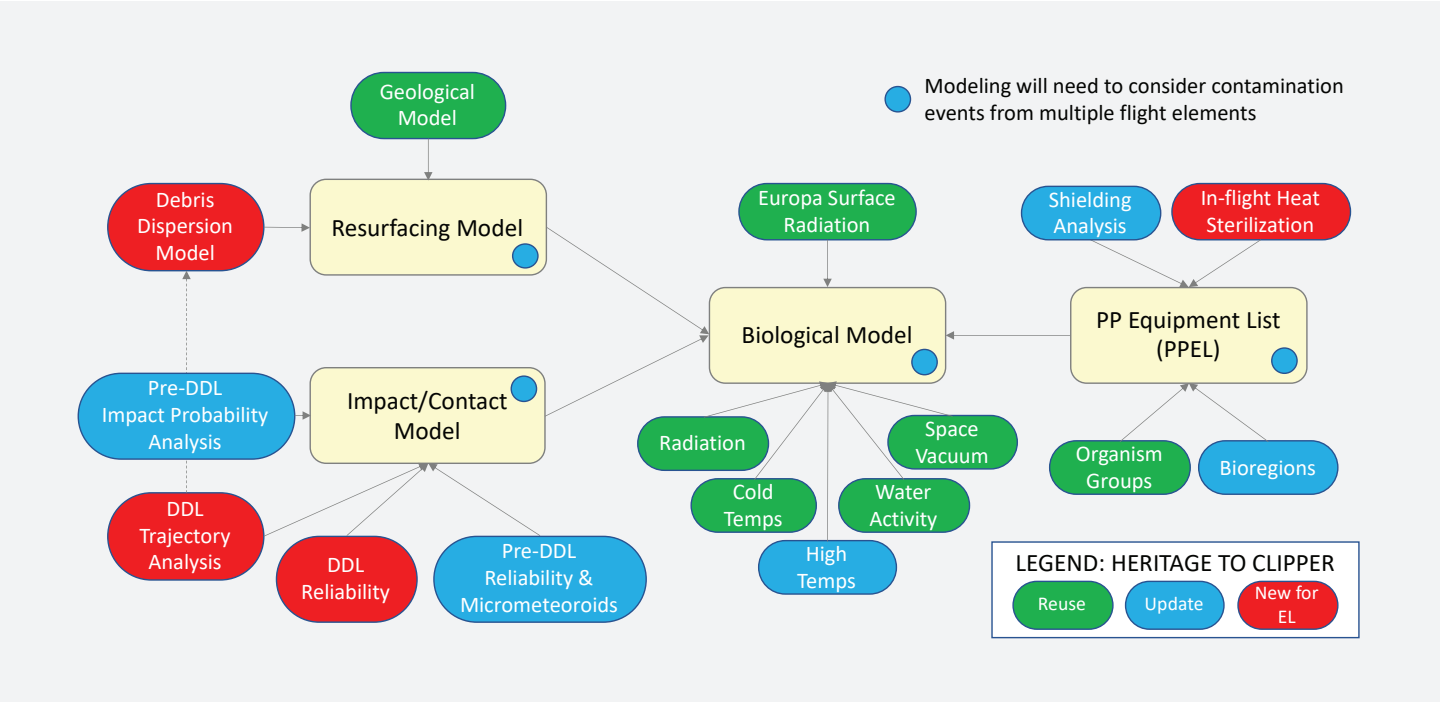

Figure 39: *Network of Submodels and Analyses Required for the Europa Lander Probability of Contamination Model*
Note: Color coding shows heritage to the Europa Clipper model.

Verification & Validation (V&V)

Once a probability of contamination model is established, calculations should be verified and results validated. Before performing V&V, *NPR 7150.2, NASA Software Engineering Requirements*[178] should be reviewed to understand the software classification of the PP model and scope the V&V appropriately. Software Quality Improvement and Software QA groups should also be involved when classifying software. Software developments should be managed in a way that is consistent with the software classification. Cybersecurity should also be considered.

Calculations can be verified by performing unit, functional, and end-to-end tests of the software; it is recommended that two people perform the calculations separately, using different software. Validation of the model is typically done by performing sensitivity analysis to study model behaviors and test their reasonableness. Mathematical approximations can also be of value for verifying calculations and validating results. These approximations can be very helpful outside of the V&V effort as

[178]NASA Procedural Requirements (NPR) 7150.2, *NASA Software Engineering Requirements*, Office of the Chief Engineer, effective from March 8, 2022 – March 8, 2027

well, since they tend to be simpler models and fast to compute, allowing quick turnaround of results and testing of model behaviors. Finally, some models cannot be calculated exactly and rely on sufficiently convergent solutions. In these cases, a convergence criterion needs to be defined and implemented by the model. Part of the verification activity is to ensure this convergence criteria is met.

Configuration Management and Archiving

A system to manage and maintain configuration during the PP model development is necessary in order to efficiently develop the model in software and ensure the results from the modeling are traceable and reproducible for V&V later in the project lifecycle. A central repository for documentation and archiving of publications, reviews, and presentations is also extremely beneficial and allows for efficient file sharing and collaboration. GitHub has proven to be a very useful application for these purposes and is recommended when developing the PP model. Use of GitHub (or other similar repository) becomes more beneficial the larger the modeling and software development teams grow.

Examples of PP Modeling

The probability of contamination by missions to ocean worlds was evaluated in various ways since the discovery of these worlds in the solar system. With scientific knowledge of these worlds and terrestrial biology increasing over the last several decades, better-informed models may now assess this probability. There have also been several attempts that failed to capture key dependencies that affect this probability, which we will not focus on here.[179] Collected here are some examples of PP modeling of the probability of contamination over time, both for forward and backward contamination.

Europa Clipper Mission

A mathematically rigorous and well-documented example of a PP model implemented on a flagship mission comes from the Europa Clipper Mission, thoroughly discussed in McCoy 2021.[180] The Clipper model was developed in Phase B and used through System Integration Review (SIR) primarily to demonstrate compliance with the Level 1 PP requirement and set adequate but not excessive bioburden allocations. The approach and results were reviewed at a three-day workshop in November 2018 by a committee of experts in reliability, mission design, physics, mathematics, microbiology, PP, and the NASA OPP, after which the committee deemed the modeling approach and model results valid and credible.[181] A key result of this modeling was that compliance was demonstrated with the Level 1 PP requirements, and that extensive bioburden reduction would not be necessary, likely saving the Project tens of millions of dollars in requalification of electronics, relieving schedule pressure, and reducing overall complexity of the Project's implementation.

Europa Lander Concept

Building on the Europa Clipper model, the Europa Lander effort extended the model to a landed mission with multiple flight elements and in-flight sterilization. See DiNicola 2022[182] for further discussion of the mathematical architecture. Since Europa Lander contacts the surface of Europa as part of the nominal mission, the model needed to capture a more complex set of contamination events: not only those that occur due to off-nominal events (as in the Clipper model), but also those that come from the nominal mission (Clipper did not contact Europa as part of its nominal mission). While unique analyses need to be completed for this PP model, such as those studying solid propellant detonation, lower-velocity impacts (relative to Europa Clipper), and in-flight sterilization that could occur during deorbit or surface operations, there is significant heritage to Clipper (Figure 39). Also, this model was developed for use in pre-Phase A to make architecture trades and feed into landing site selection decisions, whereas the Clipper model was developed in Phase B and used through SIR primarily to demonstrate compliance with the Level 1 PP requirement and set bioburden allocations. In May 2022, the Europa Lander Team held a two-day peer review with the NASA OPP and a similar (but smaller) panel of experts as at the Clipper workshop to assess the validity and credibility of the modeling approach. A key result of the modeling, concurred with by the review panel,

[179] The Coleman-Sagan approach, applied in several assessments of the probability of contamination, did not capture key dependencies associated with the spacecraft trajectory and utilized linear approximations when the behavior was significantly non-linear.
[180] See footnote 162.
[181] This was documented in a Memorandum of Understanding between JPL and NASA HQ, Subject: Europa Clipper Planetary Protection Workshop Decisions and Agreements; signed April 1, 2019.
[182] See footnote 176.

was that a new technology Terminal Sterilization System and large (~300 kg) biobarrier could be descoped from the architecture, freeing up mass and financial resources to address other Project challenges. The PP modeling team also took recommendations from the panel for future action. A manuscript is currently in development for submission to *The Planetary Science Journal* to summarize the model architecture, sensitivities, and future work.

Other Previous PP Assessments

The Juno Mission, launched in 2011 and arriving at Jupiter in 2016, needed to demonstrate compliance with the same PP requirement as Europa Clipper. The modeling performed prior to launch is discussed in Bernard 2013.[183] While the mathematical modeling was less rigorous than that done for Europa Clipper and Lander, many of the same analyses were performed and similar notions incorporated. A key difference from the Europa Clipper Mission was the velocities of inadvertent impacts (typically >20 km/s), which led to vaporization of spacecraft materials, except for highly oblique impacts. Another difference was the modeling of Europa's geologic activity. A more refined model traceable to the scientific literature is used for the Europa Clipper Mission and Europa Lander PP models. The PP model for Europa Clipper was adopted for Juno when assessing their extended mission in 2019 and 2020.

The Jupiter Icy Moons Orbiter (JIMO) Mission concept also performed an initial study to assess the mission's probability of contamination.[184] Amongst other things, this study concluded that a period of biological exploration was needed in order to meet the PP requirement without having to take "special measures" with respect to spacecraft reliability and pre-launch bioburden reduction. Interestingly, the lack of sensitivity to bioburden was pointed out (see slide CK-48 of Kohlhase 2004a[185]), although not addressed in as much detail as for Europa Clipper (see *Section A5.4.0 Development of the PP Model*).

Key Behaviors of the Probability of Contamination

This section describes two behaviors of the probability of contamination modeling for practitioners to keep in mind as they perform analyses and scope work. First, the lack of sensitivity of the probability of contamination to pre-launch bioburden reduction is discussed. This result typically comes across as non-intuitive to others, but the reasons for this are explained herein, with an example taken from Europa Clipper and Europa Lander. Secondly, parameters related to resurfacing and debris modeling are discussed. Not only is the probability of contamination highly sensitive to them, but the uncertainty inherent in these parameters contributes significantly to the uncertainty in the probability of contamination.

Sensitivity of the Probability of Contamination To Pre-launch Bioburden Reduction

Figure 40 shows the Europa Clipper probability of contamination with respect to orders of magnitude reduction on its pre-launch bioburden. For Clipper, the at-launch viable bioburden count is estimated to be $N_0 \approx 10^{13}$, absent any bioburden reduction. The CBE estimate for bioburden at launch ($N_0 \approx 10^{10}$) assumes that all hardware has received a 3-order microbial reduction and the best-case bioburden ($N_0 \approx 10^{7}$) applies a total of 6-order reduction to initial estimates. Notice the relative plateau for the probability of contamination (shown in log scale) at all orders of magnitude reduction up to approximately 12. Beyond this, the probability of contamination drops off precipitously. Although it may intuitively seem that investing in pre-launch bioburden reduction measures or leveraging the gradual death of microorganisms in flight or on the surface of Europa will equate to progress toward requirement compliance, this is not the case for Europa Clipper. The fundamental reason for this is that contamination is defined as the delivery of a single viable microorganism into a liquid water environment, something very difficult to achieve.

Despite the likelihood of any one microorganism dying being close to unity, the likelihood of every single microorganism dying is still close to zero. Since microorganisms are treated as independent (once conditioned on trajectory, debris, and resurfacing characteristics), the probability that one or more survive is governed by the expression:

$$1 - (1 - s)^{N_0}.$$

Here, s is the probability that an individual microorganism survives, so 1-s is the probability that an individual microorganism dies. Therefore, $(1 - s)^{N_0}$ is the probability that all N_0 microorganisms launched on the spacecraft die, and so the probability

[183]Bernard D, Abelson R, Johannesen J, Lam T, McAlpine W, Newlin L, *Europa planetary protection for Juno Jupiter Orbiter*, Advances in Space Research, 52, 547-568, 2013
[184]Kohlhase, *Meeting the Planetary Protection Requirement for JIMO/Europa*, slides presented to the JINO SDT, May 13, 2004a
[185]See footnote 183.

that one or more microorganisms survive is equal to $1 - (1 - s)^{N_0}$. Note that, even when s is very small (e.g., a microorganism has a 10^{-6} chance of surviving), with a bioburden as large as that on Clipper, the probability one or more survive will be close to a value of one. Because this behavior applies even to the most shielded area of the spacecraft, P_c plateaus until all organisms in all spacecraft areas have a sufficiently high probability of death. Put another way, one hardware element on the spacecraft can bottleneck the probability of contamination for the mission. For Europa Clipper, heavily shielded areas such as those on the vault and actuators associated with the solar arrays would not experience significant radiation and many times little impact heating for maneuvers that impact at highly oblique angles shortly after loss of maneuverability. For a more in-depth discussion of this behavior for Europa Clipper's probability of contamination, see McCoy et al 2021.[186]

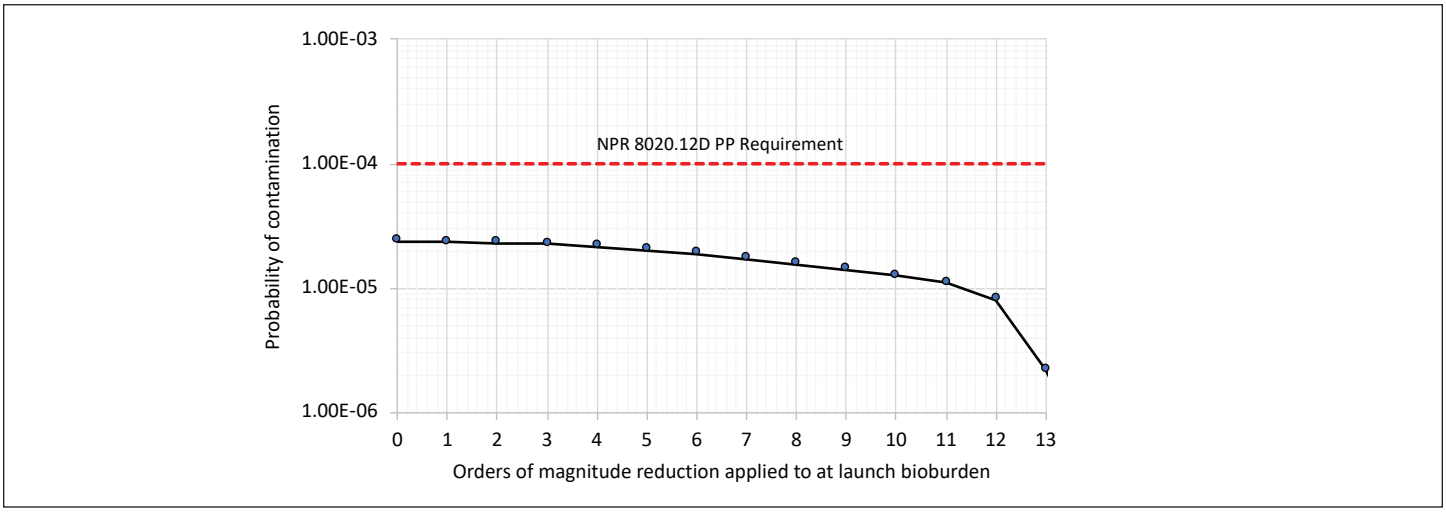

Figure 40: *Probability of Contamination as a Function of Order-of-Magnitude Reductions in Bioburden Prior To Launch*
Note: Heat microbial reduction protocols allow a 6-log reduction credit maximum. Figure reproduced from McCoy 2021.

Another way to illustrate how this can happen is shown in Figure 41. Imagine there is a distribution of bioburden with expected resistance value at the value shown in the Clipper PPEL (red vertical line), such as the distribution on the right side of Figure 41. The probability that there are zero microorganisms is vanishingly small as is evidenced by the left tail dropping off very far from zero. If a 6-order bioburden reduction process is applied, the distribution shifts to the left with expected value reducing by six orders of magnitude, but the left tail remains far enough from zero so that the probability that the bioburden is zero is still extremely small. The distribution does not approach zero until about 11 orders of magnitude bioburden reduction are applied. For Europa Clipper, the PP requirement is satisfied when this distribution has more than 99.99% of the area under the curve at zero viable microorganisms.

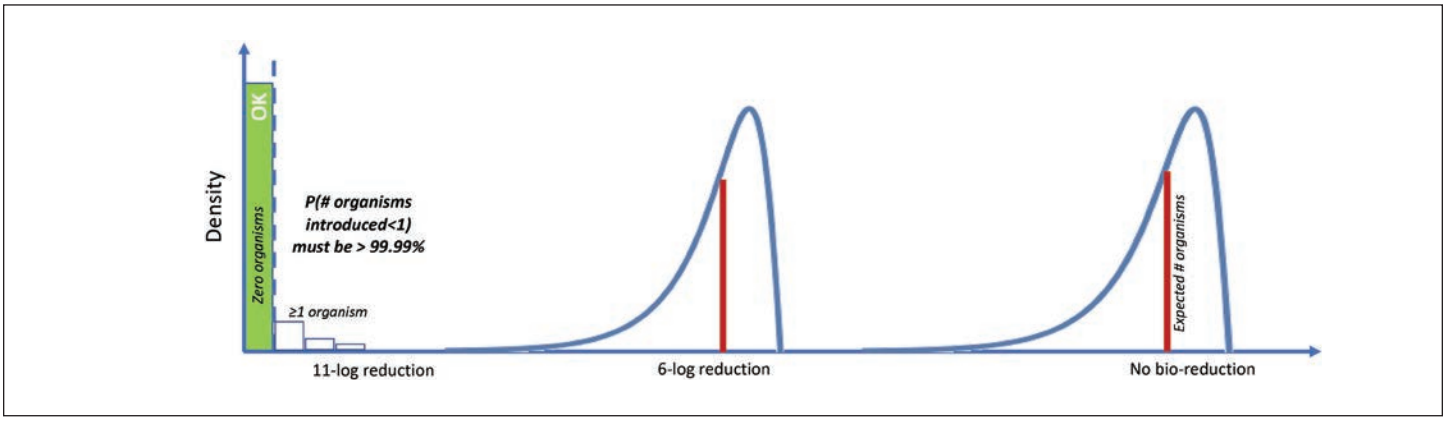

Figure 41: *Illustrative Example of the Effect of Bioburden Reduction on the Probability of Contamination*

[186]See footnote 162.

This general non-linear shape of the curve in Figure 40 is the same for any mission or study calculating a probability ≥ 1 microorganisms survive. Take, for instance, the probability of contamination of Europa Lander's nominal mission with respect to order of magnitude reductions in bioburden, shown in Figure 42. The same plateau can be seen, although shorter-lived, than that seen for the Europa Clipper analysis. In this case, ~6-7 orders of magnitude reduction in pre-launch bioburden need to be completed before significant drops in the probability of contamination are realized. However, as off-nominal scenarios are incorporated into the probability of contamination calculation (not shown in the figure), the plateau extends further to the right before the probability of contamination starts to decrease significantly. This is because off-nominal scenarios would tend to produce inadvertent (and uncontrolled) impacts of flight elements, leading to significant amounts of unsterile debris being scattered over a wider portion of the Europan surface, with less time to become sterilized before a resurfacing event brings a microorganism delivered by this process into the subsurface.

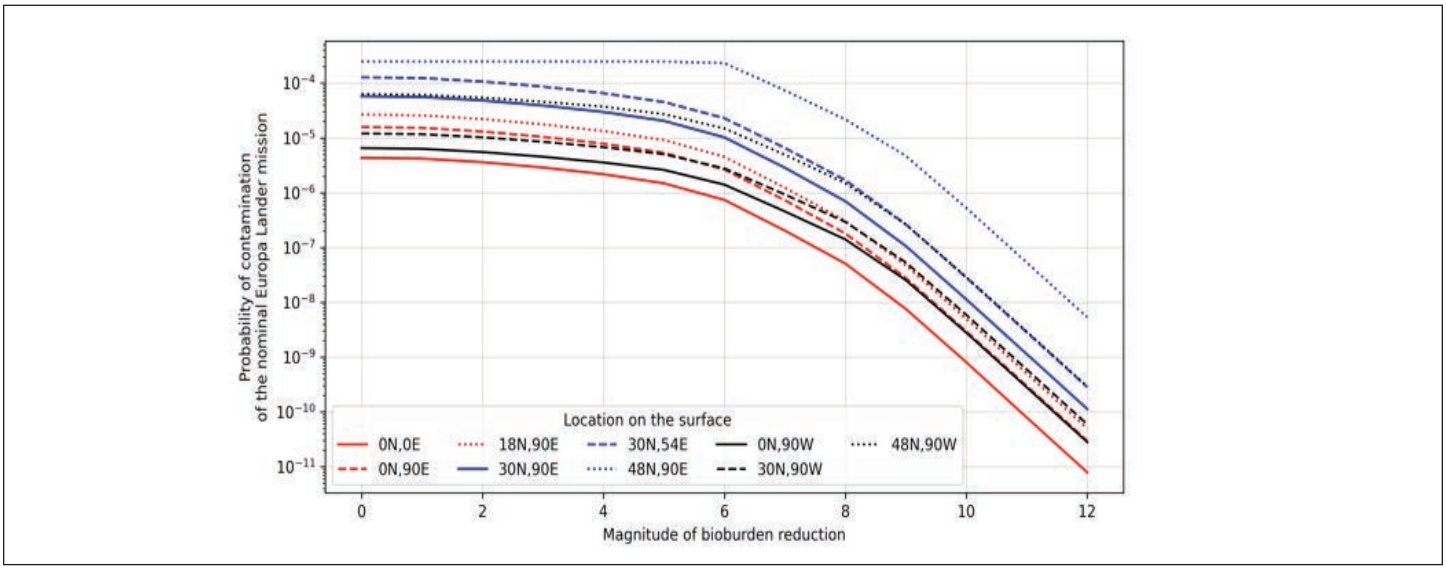

Figure 42: *Probability of Contamination of the Nominal Europa Lander Mission as a Function of Order-of-Magnitude Reductions in Bioburden*
Note: Different curves represent different landing site locations.

Sensitivity of the Probability of Contamination To Resurfacing and Debris Model Parameters

Figure 43 shows the top 10 most influential parameters affecting the probability of contamination for the Europa Clipper Mission. The top five of these all have to do with debris/fragmentation of the spacecraft and resurfacing of Europa after an impact of the spacecraft. These parameters have large ranges due to uncertainty in the environment that will not be resolved until missions such as Clipper investigate Europa and return data, or additional research is performed to constrain the parameters. Notably, the probability of contamination is relatively insensitive to the initial bioburden, which comes in as the eighth most influential parameter (see *Section A5.6.0 Key Behaviors of the Probability of Contamination* for further discussion).

While spacecraft break-up and debris dynamics are complicated and alter with impact speed, obliquity, and material types, similar assessments on Europa Lander (which has much slower impact speeds than Europa Clipper) have shown similar driving parameters for the probability of contamination. Furthermore, the geological activity of the ocean world being investigated plays a fundamental role in how influential or relevant these parameters are. Current scientific understanding of geological processes on Europa, Ganymede, and Enceladus, all targets of interest to current and future NASA missions, involve some notion of resurfacing, so these models and parameters will remain relevant for the foreseeable future.[187]

Finally, while not shown in Figure 43, the period of biological exploration is also a very influential parameter in the probability of contamination model. Decisions as to the value of this parameter are policy-driven, with the current COSPAR value set at 1,000 years,[188] but sensitivity to this parameter should be kept in mind for future assessments and discussions between the project and the PP authority at NASA.

[187]It should be noted that, at least for Enceladus, plumes are currently active and would involve additional modeling not yet developed at the time this handbook was written.
[188]See Footnote 26.

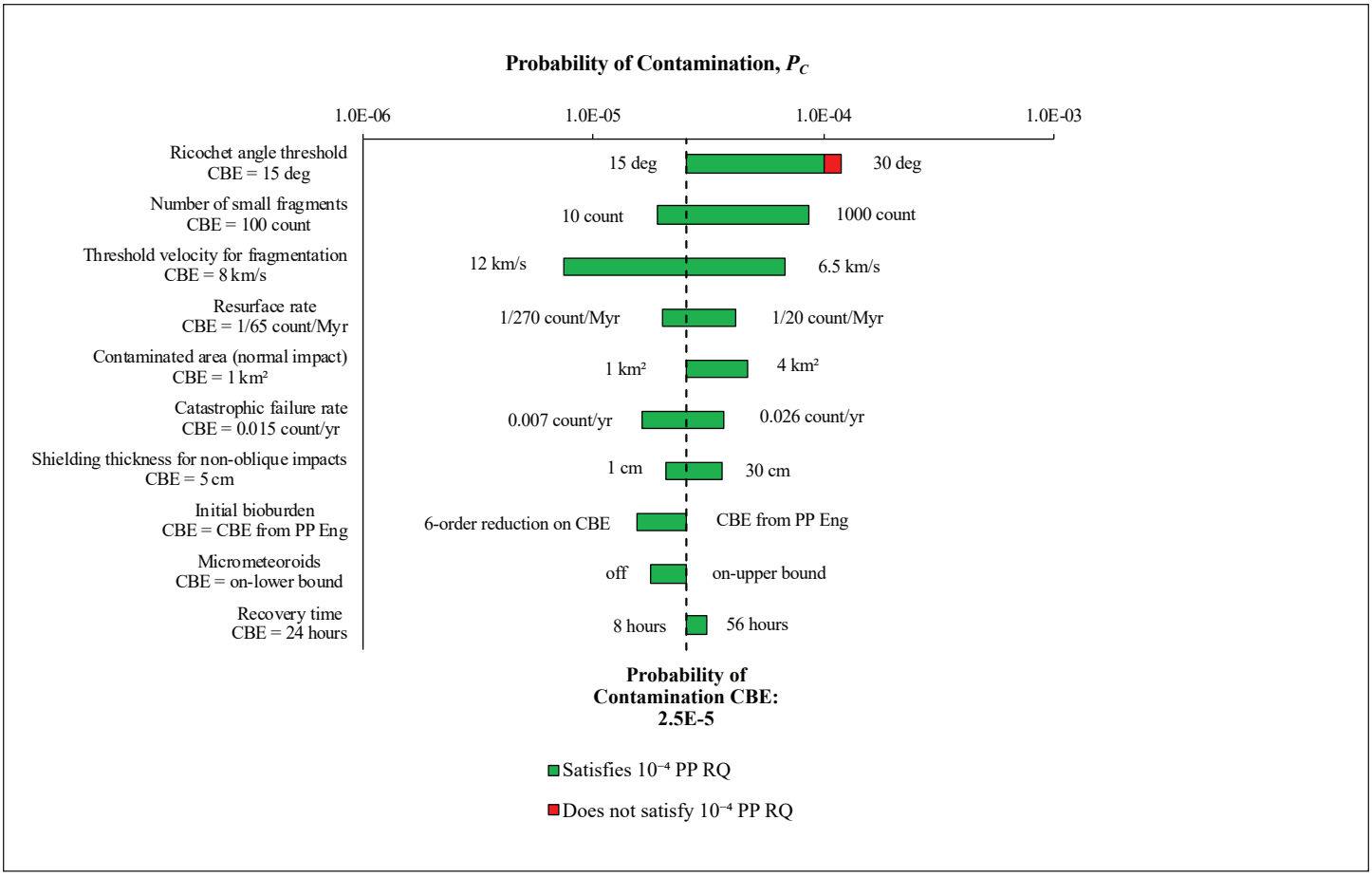

Figure 43: *Tornado Chart Showing the Effect on the Overall Probability of Contamination of Europa From the Europa Clipper Mission Note: Mission was evaluated at CDR when varying one single model parameter at a time to its best-case and worst-case values. The green portion of each bar signifies that the PP requirement is satisfied, while the red portion signifies that the PP requirement is not satisfied.*

Addendum: Key Concepts When Formulating the Mathematical Model

This section discusses some of the critical notions and principles underlying most mathematical model developments, including the formulations of the probability of contamination, denoted by P_c. Notions such as conditional probability and mutual exclusivity are defined and briefly discussed. Principles behind how a physical or phenomenological model can be brought into a probabilistic architecture (de Finetti's theorem) and how the parameters of this model can be informed by data (Bayes' theorem) are outlined with references to textbooks for further study. First, some mathematical notations are introduced.

Notation

Section A5.2.0 Acronyms, Definitions, and Notation contains a summary of the mathematical notation used in this appendix, which is discussed further here. The notation $p(A \mid B)$ is used to denote the conditional probability function of an event A given another event B. Writing $p(\mathrm{A})$ denotes the probability function when the event A is conditioned on the entire set of possible outcomes and this set of outcomes is understood. This is sometimes referred to as the "unconditional" probability function.

Similarly, the notation $p(x \mid y)$ is used to denote the conditional probability function of a random variable (or random vector) X taking the value x given another random variable (vector) Y takes the value y. When X is discrete, $p(x \mid y)$ is understood to be a probability mass function; when X is continuous, $p(x \mid y)$ is understood to be a probability density function. The unconditional probability function of a random variable (vector) X is denoted as $p(x)$. The notation $p(X \mid Y)$, $p(X)$ is used to denote these probabilities as functions of the random variables rather than as functions evaluated at specific values of the random variables.

Note that, even though there can be subtle differences in notation and terminology, both events and random variables describe sets of outcomes of interest; one just may be more practical than another. But it should be kept in mind that events can be written as random variables and random variables can be written as events.[189]

Key Notions From Probability Theory: Conditional Probability, Independence, and Mutual Exclusivity

The notion of *conditional probability* is fundamental to building probabilistic models and is a fundamental notion to probability theory itself. Conditional probability acknowledges assumptions made in models and ensures that key dependencies are considered in probability calculations. Mathematically, the conditional probability of event A given another event B is written $p(A \mid B)$ and can be calculated as $p(A \mid B) = \frac{p(A \cap B)}{p(B)}$. Note that $p(A \cap B)$ is the probability both events A and B occur together, and $p(B)$ in the denominator renormalizes the probability as if the event B has occurred. The event B may give us additional information that affects our assessment of the probability of A.

One of the extremely useful aspects of conditional probability is the following: for events $A_1,A_2,...,A_n$,

$$p(A_1 \cap A_2 \cap ... \cap A_n) = p(A_1) \times p(A_2 \mid A_1) \times ... \times p(A_n \mid A_1,...,A_{n-1});$$

that is, a complex or unknown joint probability distribution can be expanded into a product of single event conditional probability distributions that are known or more readily discerned.

When the event B does not give us additional information that affects the probability of A, the two events are referred to as independent; that is, $p(A \mid B) = p(A)$. Observe that when a set of events $A_1,A_2,...,A_n$ are mutually independent of one another, the probability that all of these events occur is the product of the individual probabilities; that is, $p(A_1 \cap A_2 \cap ... \cap A_n) = \prod_{i=1}^{n} p(A_i)$.

Another fundamental notion underlying probabilistic models is that of *mutual exclusivity*. Two events A and B are mutually exclusive when they have no outcomes in common, and so $p(A \cap B) = 0$. For instance, if A is the event that a spacecraft is on trajectory T_1 (a set of coordinates identifying the time and location of the spacecraft) and B is the event that the spacecraft is on a different trajectory T_2, then the probability that the spacecraft is on both trajectories is zero. Observe that when a set of events $A_1,A_2,...,A_n$ are mutually exclusive from one another, the probability that one or more of these events occur is the sum of the individual probabilities; that is, $p(A_1 \cup A_2 \cup ... \cup A_n) = \sum_{i=1}^{n} p(A_i)$.

Key Theorems for Probabilistic Models: de Finetti's and Bayes' Theorems

A scientist observing some phenomenon is compelled to understand how the phenomenon occurs, and therefore how it can be explained given our current knowledge of processes that take place in the physical world. At some point, whether it be the development of a new theory, the strength of analogy to another phenomenon, or additional data being acquired, the scientist hypothesizes what is referred to as a *phenomenological model* that attempts to account for the phenomenon observed and the processes that led to its occurrence.[190] This model utilizes relationships from quantitative disciplines such as physics, thermodynamics, or chemistry, often described by differential equations. The scientist then uses this model to predict the outcome of an experiment or validate certain hypotheses.

Typically, at some point in the scientist's inquiry, the model is found to be insufficient to explain the data observed. The scientist's knowledge of the phenomenon is uncertain, and quantifying this uncertainty becomes part of the model development process. The importance of this UQ depends on the application, decisions being made from the model, and tolerance for risk. But to do this, the phenomenological model must be integrated with principals of probability.

The bridge between phenomenological and probabilistic models is crossed using what is known as de Finetti's theorem. This will involve fundamental notions from probability theory, such as independence, exchangeability, and uniformity (i.e., being identically distributed). The resulting model will require statistical analysis to calibrate and validate with data. Bayes' theorem provides the rigorous foundation for this analysis.

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[189]An event E is a subset of outcomes from the set of all possible outcomes. An event E with a defined probability can be written as the random variable $X(\omega)=1$ if ω is an outcome in E; $X(\omega)=0$ otherwise. Conversely, if X is a random variable with measurable probability, then the event that X takes the value x is the set of outcomes $E=\{\omega \mid X(\omega)=x\}$.

[190]This model might use physical laws (e.g., Newtonian physics) or some empirical relationship determined through experiment (e.g., Arrhenius equation), or both.

It should be noted that the intellectual flow described above can move in a somewhat opposite direction as well. For instance, a statistician may observe some phenomenon and, being compelled initially to predict (rather than explain, as in the case of the scientist above), develop a statistical model using probability distributions and little else. How credible this model is when applying it to real-world scenarios (e.g., answering scientific questions, determining risk to mission success) that push outside the data range used to inform the model's parameters (extrapolation) tends to be where these models fall short. This statistician finds themselves in need of some phenomenological modeling in order to draw credible predictions, especially when extrapolating. Hence, there is a convergence of intellectual processes to a model that balances both phenomenological and statistical uncertainties in a way that is appropriate for the given application. Indeed, this should be the goal of any applied modeling effort. The following sections articulate the mathematical justification and approach for doing this rigorously. While these models can be computationally intensive or even intractable, approximations can be pursued that can be implemented once the theoretical model is articulated.

A concise version of de Finetti's theorem can be stated as follows: for any sequence of exchangeable[191] random variables $X_1, \ldots, X_n$, there exists a conditioning event E such that

$$p(X_1, \ldots, X_n \mid E) = \prod_{i=1}^{n} p(X_i \mid E);$$

that is, if you can find the right conditioning event, then you can treat the random variables as independent if you condition on that event.

Now, the phenomenological model M is the result of an attempt to find all observable variables Y and how they relate with one another to determine the observable outcome X of a process of interest. With most models come unobservable variables $\boldsymbol{\Theta}$, which we will refer to as parameters, that typically quantify the effect of observable variables on the process. The model M relates the terms X, Y, and $\boldsymbol{\Theta}$ by way of some function or set of functions and is a mathematical representation of our knowledge that attempts to account for the phenomenon being studied. Put another way, there should be no more information Z that can be added that would change our state of knowledge — or probability — of the outcome $X = (X_1,\ldots,X_n)$; or more formally, $p(X_1, \ldots, X_n \mid \boldsymbol{\Theta}, Y, M, Z) = p(X_1, \ldots, X_n \mid \boldsymbol{\Theta}, Y, M)$. This is just saying that, if the random variables being evaluated are exchangeable, our phenomenological model posits that we have found an event guaranteed to us by de Finetti's theorem, this event being defined by the values of $\boldsymbol{\Theta}$ and Y when used in model M, and

$$p(X_1, \ldots, X_n \mid \boldsymbol{\Theta}, Y, M) = \prod_{i=1}^{n} p(X_i \mid \boldsymbol{\Theta}, Y, M);$$

The veracity of this assertion is tested when the probabilistic model is validated.

With the phenomenological model placed in a probabilistic framework, parameters $\boldsymbol{\Theta}$ need to be informed by n observed data points $x_1, \ldots, x_n$ (a realization of $X_1, \ldots, X_n$). This can be done in a statistically rigorous manner using Bayes' theorem. Stated in its most basic form, for any two events A and B where $p(B)>0$

$$p(A \mid B) = \frac{p(B \mid A) \times p(A)}{p(B)}$$

$$\propto p(B \mid A) \times p(A).$$

Note that since $p(B)$ is a constant in this calculation, Bayes' theorem can be written as a proportion omitting $p(B)$, as shown on the second line of the expression above. This form tends to be more useful for computations.

From the preceding discussion related to the phenomenological model M, Bayes' theorem can be used to find the probability that the parameter $\boldsymbol{\Theta}$ takes the value Θ given the observed data, observables, and model (i.e., the posterior distribution):

$$p(\Theta \mid x_1, \ldots, x_n, Y, M) = \frac{p(x_1, \ldots, x_n \mid \Theta, Y, M) \times p(\Theta \mid Y, M)}{p(X_1, \ldots, X_n \mid Y, M)}$$

$$\propto [\prod_{i=1}^{n} p(x_i \mid \Theta, Y, M)] \times p(\Theta \mid Y, M),$$

[191] By exchangeable it is meant that $p(X_1 = x_1, X_2 = x_2, \ldots, X_n = x_n) = p(X_1 = x_{\sigma(1)}, X_2 = x_{\sigma(2)}, \ldots, X_n = x_{\sigma(n)})$, σ is any permutation of the indices $\{1,\ldots,n\}$; that is, the joint probability is the same no matter how we swap around (exchange) the values taken by the random variables.

where the conditional independence gained from de Finetti's theorem is used in the second line of the equation. Here, $p(\Theta \mid Y,M)$ is often referred to as the prior distribution of parameters Θ (that is, the probability distribution prior to considering the observed data), and $p(\Theta \mid x_1, \ldots, x_n, Y, M)$ is referred to as the posterior distribution of parameters Θ (that is, the probability distribution after considering the observed data). There are many options for prior distributions, but it is recommended that all information available that does not depend on the observations $x_1, \ldots, x_n$ be used when constructing this probability distribution. When no credible, quantifiable information exists, a Jeffreys Prior or reference prior is recommended to convey ignorance of the parameter values. These prior distributions convey ignorance by maximizing the expected information gained by the data using the notion of Kullback-Leibler divergence.[192] Note that typically the posterior distribution must be estimated using Markov Chain Monte Carlo (MCMC) methods and the proportionality above from Bayes' theorem.

From here, just about any quantity can be probabilistically estimated that is a function of observables Y and parameters Θ, given a model M. Letting $Q(\Theta,Y) = q$ be the event that the quantity of interest takes the value q when evaluated at Θ and Y, the probability function associated with the quantity of interest, given the model and observed data, is given by

$$p(q \mid x_1, \ldots, x_n, Y, M) = \int_{\Theta} p(Q(\Theta,Y) = q \mid M)\, p(\Theta \mid x_1, \ldots, x_n, Y, M)\, d\Theta .$$

This equation follows from the definition of conditional probability, mutual exclusivity of parameter values on disjoint intervals of size $d\Theta$, and the assumption that the quantity of interest Q is independent of the observed data $x_1, \ldots, x_n$ once Θ,Y and M are known. This equation typically requires MCMC simulation to compute.

For further reading on Bayesian analysis and probability theory, please see Gelman 2014,[193] McElreath 2019,[194] and Chung 2001.[195]

[192]Berger J, Bernardo J, Dongchu S, The Formal Definition of Reference Priors, The Annals of Statistics, Vol. 37, No. 2, 905-938, 2009
[193]Gelman, Andrew, *Bayesian Data Analysis, Third Edition*. Chapman & Hall, Taylor & Francis Group, LLC, 2014
[194]McElreath, Richard, Statistical Rethinking, Second Edition. 2019
[195]Chung, Kai Lai, *A Course in Probability Theory, Third Edition*. Academic Press, San Diego, 2001

Appendix 5: Generalized Mars Mission 50-Year Probability of Impact Requirement Compliance

Executive Summary

This appendix provides a comprehensive overview of the methodology and requirements for PP compliance for interplanetary missions. Specifically, the focus is on the upper stage of the launch vehicle, which must have a less than 1 in 10,000 chance of impacting Mars within 50 years after launch. This chapter details the sequence of events involved in the upper stage's trajectory and identifies the various factors that can cause an impact, including resonant trajectory and encounters with other planetary bodies.

A statistical formulation is provided for calculating the probability of impact based on the success or failure of each event in the upper stage sequence. This appendix emphasizes the importance of considering both the nominal mission scenario and potential anomalous scenarios when assessing the impact probability. Overall, this appendix provides a technical overview of the complex process involved in ensuring compliance with PP requirements for interplanetary missions.

Introduction

PP requires all interplanetary missions' launch vehicles' upper stage to have a less than 1 in 10,000 chance of hitting Mars within 50 years after launch. In order to ensure this requirement, a set of rules and methodologies was developed during InSight and Mars 2020 Projects and should serve as a reference for other missions and institutions.[196,197]

Background

The sequence of operations that occur after launch is mission-dependent; however, typically the event consists of the following steps:

(i) Separation of the upper stage at a certain altitude
(ii) Deceleration
(iii) CCAM
(iv) Deceleration
(v) Discard of the leftover LOX/LH2 and hydrazine (known as "the blowdown")

Each of these steps determines the probability of the impact. To ensure PP compliance, it is important to consider both the nominal mission scenario and any potential anomalous scenarios, such as failure to separate, failure to perform the CCAM, or failure to perform the blowdown.

No matter if the separation-CCAM-blowdown sequence is finished or aborted, the upper stage will fly toward Mars. The initial uncertainty of the spacecraft's state is presented using a 6 x 6 injection covariance matrix (ICM), which is then used to map the time of closest approach via state transitions matrix. The resulting hyper-ellipsoid is then projected into the Mars B-Plane. To meet mission objectives, the spacecraft must overcome the "launch bias" caused by the design of the Earth departure hyperbola. This is achieved by designing the separation, CCAM, and blowdown altitudes to move the first encounter with Mars further away from it.

However, there are three factors that can still cause the upper stage to impact Mars. The first is resonant trajectory, where the upper stage's orbit period matches a rational fraction of Mars' year. Second, the post-encounter orbit could be in the same plane as Mars, leading to an impact anywhere in Mars' orbit. Lastly, later encounters with other planetary bodies, like Earth, could change the upper stage's trajectory and result in an impact.

Workflow

Steps and procedures described below were developed during previous missions to Mars, including InSight and Mars 2020 Projects, and are intended to serve as a reference for future missions.

[196]Wallace, M. S. 50-Year Probability of Impact Requirement Compliance, IOM InSight-4800-18-002. (2018)
[197]Wallace, M. S. 50-Year Probability of Impact Requirement Compliance, IOM M2020-630-20-069. (2020).

Statistical Formulation for the Upper Stage Sequence

The probability of impact $P(I)$ can be expressed as the product of the probabilities of occurring events. The probability of a successful upper stage/spacecraft separation (s), successful CCAM (c), and successful blowdown (b) are denoted as $P(s)$, $P(c)$, and $P(b)$, respectively, with the failure of each indicated as $P(\overline{s})$, $P(\overline{c})$, and $P(\overline{b})$, where the probability of failure is denoted as one minus the probability of success; e.g., $P(\overline{s}) = 1 - P(s)$. Additionally, the $P(I)$ given that event (s) has occurred is denoted as $P(I \mid s)$, while the $P(I)$ given that events (s) and (b) have occurred is denoted as $P(I \mid s,b)$; therefore, the total $P(I)$ can be expressed as:

$$\begin{aligned} P(I) = \; & P(\overline{s})P(I \mid \overline{s}) + \\ & P(s)P(\overline{c})P(I \mid s,\overline{c}) + \\ & P(s)P(c)P(\overline{b})P(I \mid s,c,\overline{b}) + \\ & P(s)P(c)P(b)P(I \mid s,c,b) \end{aligned}$$

1. The impact probability, given some sequence of events [the (z)], can be divided into two parts: the probability of impact on the first encounter [the $P(I_0)$] and the probability of impact in the next 50 years [$P(I_{50})$], given no impact occurred in the first encounter.

$$P(I \mid z) = P(I_0 \mid z) + P(\overline{I}_0 \mid z)P(I_{50} \mid \overline{I}_0, z)$$

2. The total impact probability total [$P(I)$] can be expressed by combining Equations 1 and 2 and expressed as follows:

$$\begin{aligned} P(I) = \; & P(\overline{s})[P(I_0 \mid \overline{s}) + P(\overline{I}_0 \mid \overline{s})P(I_{50} \mid \overline{I}_0, \overline{s})] + \\ & P(s)P(\overline{c})[P(I_0 \mid s,\overline{c}) + P(\overline{I}_0 \mid s,\overline{c})P(I_{50} \mid \overline{I}_0, s,\overline{c})] + \\ & P(s)P(c)P(\overline{b})[P(I_0 \mid s,c,\overline{b}) + P(\overline{I}_0 \mid s,c,\overline{b})P(I_{50} \mid \overline{I}_0, s,c,\overline{b})] + \\ & P(s)P(c)P(b)[P(I_0 \mid s,c,b) + P(\overline{I}_0 \mid s,c,b)P(I_{50} \mid \overline{I}_0, s,c,b] \end{aligned}$$

3. The problem of determining the total impact probability for a given design can be reduced to finding each individual term.

Probability Analysis

Reliable assessment of the probability of impact after many years requires a Monte Carlo simulation. Conventional methods, including ICMs, to determine impact probabilities are not effective when large and uncertain changes in velocity (ΔV) occur, such as during planetary encounters. Over time, uncertainties can become non-Gaussian, even without a subsequent flyby.

Monte Carlo simulations involve multiple trials, leading to estimates of probabilities that have their own uncertainty. The simulation results in either a flyby or impact with some true probability, which can be modeled as a Bernoulli process. The uncertainty follows a Beta distribution, and the larger the Monte Carlo simulation, the smaller the uncertainty.

The true reliability of the upper stage, and thus the probability of different scenarios, can also be treated as a Bernoulli process, based on the stage's flight record or estimated reliability rather than a Monte Carlo simulation (Figure 44 and Figure 45).

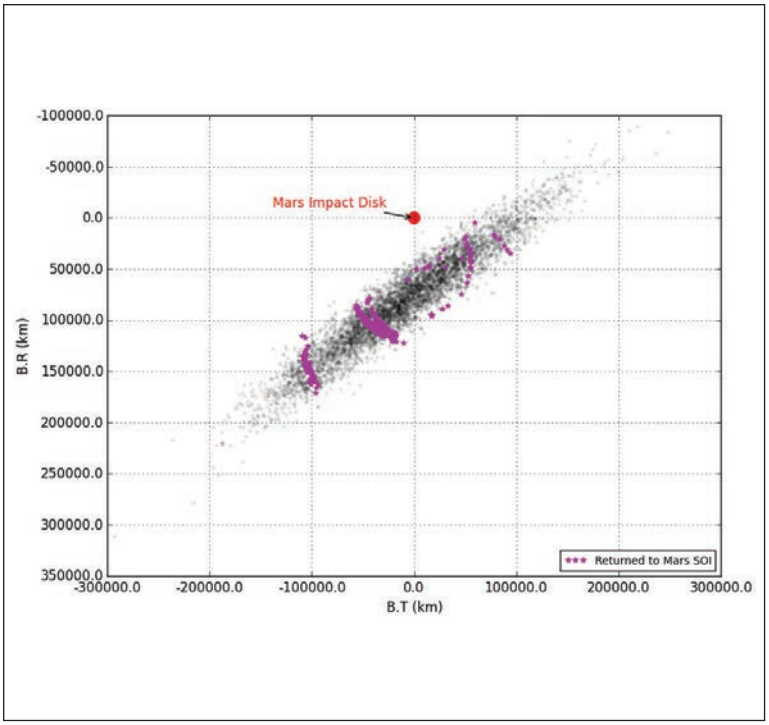

Figure 44: *Direct Resonance Scenario*
Note: 256 of 5,000 predicted cases returned to Mars' sphere of influence (SOI), which spans over 574,000 km; however, no impacts were predicted. B.T / B.R – B- is a vector defined by unit vectors T and R.

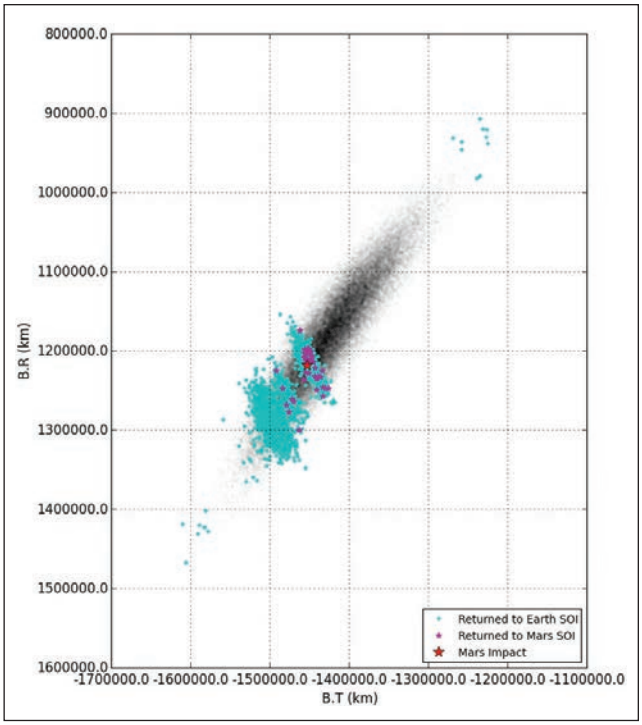

Figure 45: *Assisted Returns Scenario*
Note: The figure presents 50,000 Monte Carlo simulations. In this scenario, many cases predicted the spacecraft's return to the Earth's SOI, few returns to Mars SOI, and only one case predicted impacting Mars. B.T / B.R – B- is a vector defined by unit vectors T and R.

Total Probability of Impact Process

Solving Equation 3 involves a combination of analytical calculations, the upper stage flight record, and Monte Carlo propagations and involves a 5-step process:

1. ***Impact Probabilities:*** Determine the impact probabilities for each of the four nominal and failure scenarios.
2. ***50-Year Monte Carlo Simulations:*** Conduct 50-year Monte Carlo simulations for each of the four scenarios and record the cases that impact Mars.
3. ***Beta Distributions:*** Generate one million samples of each of the seven Beta distributions, which represent the three failure points of the launch vehicle and the resulting probability of four impact scenarios (nominal and failure).
4. ***Solving Equation 3:*** Use the samples from the seven sets of Beta distributions and the results from Step 1 to solve Equation 3 one million times.
5. ***99th Percentile:*** Sort the results and determine the 99th percentile value, which represents the likelihood of a successful outcome.

Data Sources

In the analysis, it is important to clearly specify all data sources that are used, including ICM and upper stage reliability. It is crucial to have a clear understanding of these data sources to ensure the accuracy and validity of the results.

Dynamic Modeling

The minimum requirement for trajectory propagation is the use of a gravity and solar radiation pressure (SRP) acceleration model. This model must take into account the point mass accelerations for the eight planets in the solar system, the Moon, Pluto, and Phobos and Deimos, the two Martian moons. Additionally, a truncated spherical harmonic field to account for the non-spherical effects of the Earth and Mars must also be incorporated.[198,199]

[198]Wallace, M. S. A Massively Parallel Bayesian Approach to Planetary Protection Trajectory Analysis and Design, AAS/AIAA Astrodynamics Specialist Conference. in (2015).
[199]Bottke, W. F., Vokrouhlický, D., Rubincam, D. P. & Nesvorný, D. THE YARKOVSKY AND YORP EFFECTS: Implications for Asteroid Dynamics. Annu. Rev. Earth Planet. Sci. 34, 157–191 (2006).

The SRP effects are based on the shape and reflectivity model and mass being propagated. The solar pressure model used assumes a pair of flat plates, equivalent to an end-over-end spinning upper stage with an unknown rate and fixed spin axis. The cylindrical model is divided into two flat planes: one always facing the Sun and the other with a randomly chosen orientation from a uniform unit sphere, which is fixed throughout the simulation.

A key assumption in the formulation is that the upper stage has no reflective component. The upper stage (e.g., Centaur) is considered to be matte, and any specularity is assumed to be worn away due to micrometeoroid impacts over the course of the simulation.

The Sun plate represents the SRP component from the spinning cylinder with the spin axis perpendicular to the sunline. The cylinder alternates between pointing its endcaps and body toward the Sun, which is the smallest area for a flat-spinning cylinder and results in an average acceleration away from the Sun. This is because the spin rate is assumed to be much faster than the orbital rate, causing the non-radial acceleration to be dispersed.

The random plate component of the SRP represents the SRP effect on the cylinder when its spin axis is not perpendicular to the Sun. This acceleration has components in different directions, depending on the orientation of the spin axis. The reflective area of this flat plate takes into account the area of the Sun plate, so that when the spin axis is parallel to the Sun, the correct total reflective area is calculated.

The model for the upper stage and spacecraft considers it as a cylinder with different reflective areas and properties on its nose, tail, and axial sides. The areas are calculated by summing and dividing by four, consistent with JSC's Debris Assessment Software's tumbling-area algorithm.[200] The axial side includes two faces in this model to determine the total weighted area

$$total = {nose}/{4} + {tail}/{4} + {axial}/{2}$$

4. When modeling a combination of an upper stage and a spacecraft, the tail end of the spacecraft and the nose end of the upper stage are treated as having a value of zero, while the values of the two axial faces are added together.

Results

For each day of the launch period, the 99th percentile probability of impact at the beginning, end, and middle points of the launch window should be included. The breakdown of the probabilities of a first-encounter impact and the results of Monte Carlo simulations should also be provided, along with the probability density function (PDF) and cumulative density function (CDF).

Final Recommendations

To ensure the success of future missions to Mars and other planets, it is recommended that the following steps and procedures, developed during previous missions such as InSight and Mars 2020 Projects, be used as a reference:

1. Apply statistical formulation for the upper stage sequence to assess the probability of impact, considering the probabilities of occurring events, such as successful upper stage/spacecraft separation, successful CCAM, and successful blowdown in the case of Mars missions.
2. Divide the impact probability into the probability of impact on the first encounter and the probability of impact in the next 50 years, given no impact occurred in the first encounter.
3. Use Monte Carlo simulations for reliable assessment of the probability of impact after many years, considering uncertainties that can become non-Gaussian, even without a subsequent flyby.
4. Treat the true reliability of the upper stage as a Bernoulli process, based on the stage's flight record or estimated reliability.
5. Follow a five-step process to determine the total probability of impact, including determining impact probabilities, conducting 50-year Monte Carlo simulations, generating Beta distributions, solving equation for the total impact probability (Equation 3) using the samples from the Beta distributions and the results from determining impact probabilities, and determining the 99th percentile value, which represents the likelihood of a successful outcome.
6. Specify all data sources used in the analysis, including ICM and upper stage reliability, to ensure the accuracy and validity of the results.
7. Use a gravity and SRP acceleration model as the minimum requirement for trajectory propagation.

[200]Debris Assessment Software (DAS), **https://orbitaldebris.jsc.nasa.gov/mitigation/das.html**

Appendix 6: Example Laboratory SOPs

Swab Method for Microbiological Sampling of Spacecraft and Environmental Surfaces

Baseline requirements for sampling a surface with swabs is described in NASA-STD-8719.27 Sections 6.5.2. and 6.6.2. A more detailed protocol is found below.

Scope and Application

This standard operating procedure (SOP) is intended to be an example, and does not replace any requirements found in NASA-STD-8719.27. Each laboratory should have internal SOPs as described in the laboratory quality management plan with details specific to their equipment and process.

This assay is used to sample microbial contamination on surfaces from spacecraft, parts, cleanroom/assembly areas, or ground support equipment. This assay is often referred to as the "Standard Swab Assay Method" and is appropriate for materials that can tolerate sample collection using a damp swab. This method is limited to sample collection from surfaces no greater than 25 cm^2 per swab. Multiple swabs may be used to sample a larger surface area.

Supplies

Sample Preparation

Consumables:
- Gloves
- 70% IPA in spray bottle
- Cotton-tipped swab
- Marker
- Sterile test tubes with cap
- 50 mL pipets
- Sterile water
- Cleanroom bags and tape

Tools/equipment:
- Biological safety cabinet
- Pipettors
- Test tube rack
- Transport container
- Autoclave

Sample processing and Analysis

Consumables:
- Sterile Petri dishes
- TSA agar
- Markers
- Gloves
- Pipette tips
- 70% IPA in spray bottle
- Low-lint laboratory wipes
- Spore positive control
- Autoclave bags
- Autoclave tape

Tools/equipment:
- Tube rack
- p1000 and p10 pipettors
- Biological safety cabinet
- Ultrasonic water bath
- Vortexer
- 80 °C water bath
- 50 °C water bath
- Ice water bath
- Ice packs
- Digital thermometers
- Timer
- Incubator
- Autoclave

Sample Kit and Laboratory Preparation

Notes on aseptic technique: *Preparation of sampling supplies should be conducted using standard laboratory PPE and gloves. Any surface that touches the sample (swab, inside of test tube, water) should be sterile and only opened inside an ISO Class 5 laminar flow bench or biological safety cabinet (BSC). Gloves should be wiped with 70% IPA before working in the flow bench, and anything entering the flow bench should also be wiped before it is placed on the bench.*

When working on a HEPA-filtered flow bench or BSC, pay attention to the flow direction of HEPA-filtered air. User should avoid passing hands or tools between the airflow and open sterile containers to maintain an aseptic workspace.

Materials used in sampling kits should be compatible with cleanroom requirements [for example, only using cleanroom paper, avoiding silicone adhesives, or meeting electrostatic discharge (ESD) requirements]. Specific compatibilities should be confirmed for each project/cleanroom. Exterior surfaces of sample kits (exterior of test tube, test tube racks, markers, etc.) should be cleaned to meet cleanroom requirements where the samples are acquired.

Day Before or Day of Sampling

1. Spray flow bench/BSC work surface with 70% IPA and wipe to clean.
2. Prepare enough sterile swabs to accommodate all swab samples to be collected, plus controls and spares.
- Use commercially available absorbent swabs firmly twisted to a suggested size of 5 x 19 mm long over one end of an applicator stick.
- Swabs can be packaged and sterilized in lab or purchased pre-sterilized.
- If desired, a breakpoint can be added before sterilization to allow easier breaking after sampling.

3. Prepare enough sterile test tubes containing 10 mL each of sterile distilled water to accommodate all swab samples to be collected, including negative controls and spares.
- Add 10 mL of sterile water into each test tube and close tightly.

4. Assemble sampling kit with sterile swabs and sterile water tubes in a test tube rack.
- Package test tube rack in a cleanroom bag or other clean containers as required by project cleanroom entry requirements. Cleanroom bags can be heat sealed or sealed with cleanroom (not laboratory) tape.
- A sampling kit can be used immediately or stored in refrigerator until ready for use.

Day of Sampling

1. Prepare ultrasonic water bath.
- Fill water bath with 0.02 percent volume-to-volume of polyoxyethylene sorbitan monooleate to water.
- Set the water bath to degas for 5 minutes.

2. Turn on 50 °C water bath.
3. If samples will be heat shocked, turn on hot water bath. It takes ~2 hours to warm up to 80 °C.
4. Prepare sufficient tryptic soy agar (TSA) according to manufacturer's direction and leave to cool in 50 °C water bath. Each swab sample is plated onto four plates, and each plate uses ~20 mL of agar. When autoclaving bottles of TSA, caps should be slightly loose to prevent vacuum.
5. Ensure that the incubator is on and set to 32 °C. Incubators can be cleaned by wiping with 70% IPA as necessary.
6. Sample kits and samples can be transported in an insulated transport cooler with ice packs. Ice packs may not be needed if the sample is returned to the laboratory for processing within an hour of sampling.

Sampling

Notes on aseptic technique: *Care must be taken so that the sampling device is not contaminated by handling. Gloves must be worn while handling the swab, and after sampling the swab must be broken off into the test tube below any parts of the swab shaft that is touched by gloves. If a swab is compromised and touches any surface beyond the sampled surface, then a false positive reading could result.*

1. Sterile swabs are used to sample up to 25 cm^2 per sample. The location and size of each sample should be recorded. If desired, sampling location can be documented by photograph.
2. Once a sampling location is identified, remove a sterile swab from its container and moisten the head of the swab with sterile water in the falcon tube. Express excess moisture from the swab against the interior wall of the tube.
3. Hold the swab so that the handle makes about a 30° angle with the surface to be sampled. While moving the swab in one direction, rotate the head of the swab slowly and thoroughly over a measured 25 cm^2 surface area. Change the linear direction of the swabbing motion 90° and again swab the surface thoroughly. Complete a third coverage of the surface by again changing the direction of the swabbing motion by 135°.
4. Put the swab into sterile test tube containing 10 mL sterile water, break the swab shaft away from any flight hardware, and close the test tube for further processing.

Sampling Controls

For each 10 or fewer samples collected, also collect a "field negative" control. Follow the sample collection procedure, except instead of sampling a surface at Step 3, wave the moistened swab through the air for 2 to 4 seconds. The swab should be exposed to the air for approximately the same amount of time that it takes to collect a normal swab sample.

Create a positive control by applying a known amount of spore positive control into a test tube with 10 mL water. Process and analyze controls alongside the rest of the samples.

Transport and Storage

Transport samples to the laboratory. Samples can be stored at room temperature if processed within 1 hour, or stored at 4-8 °C and processed within 24 hours. Time of sampling and time of processing should be noted with sample records. A portable logging thermometer may be used to monitor sample temperature during longer transport.

Sample Processing

Notes on aseptic technique: *The samples should never be opened outside of an aseptic ISO 5 environment, which usually means inside a laminar flow bench or BSC. Some processing steps such as heat shock likely take place outside of an ISO 5 environment, so for these steps the samples should be tightly closed. Tools, sample containers, and gloves should be wiped with 70% IPA before entering the ISO 5 environment.*

When working on a HEPA-filtered flow bench or BSC, pay attention to the flow direction of HEPA-filtered air. User should avoid passing hands or tools between the airflow and open sterile containers to maintain an aseptic workspace.

Extraction

Both the shaking (or vortexing) and ultrasonic steps serve to release microorganisms from the surface of the swab into solution so that it can be later plated.

1. Place each test tube containing the water and the swab on a vortex mixer and vortex at maximum power for 5-6 seconds.
   - Watch for a strong "tornado" to form in each tube to indicate that the sample is thoroughly vortexed.
2. Place each test tube containing the water and the swab in an ultrasonic bath, making sure the liquid level in the bath is above the fluid level in the tubes. Samples should be suspended in the bath using flotation or a cage to keep them from touching the bottom or sides. Sonicate for 120±5 seconds.

Heat Shock (Skip heat shock for vegetative samples)

The heat shock step brings the sample to 80 +/- 2 °C and holds it there for 15 minutes. This process kills most vegetative microorganisms and selects for the hardy, heat-resistant microorganisms (mostly spores) that may also be resistant to other cleaning procedures and the extreme environments of space.

1. Place the test tubes containing the vortexed and sonicated samples in a water bath at 80±2 °C for 15 minutes, as determined by a pilot tube containing a thermometer. Make sure the liquid level in the water bath is above the fluid level in the tube.
   - *The pilot tube should be a test tube with the same volume of water in it as the rest of the swab samples and be processed along with the sample tubes so that it is the same temperature when it goes into the water bath. A calibrated thermometer is placed in the pilot tube and the 15-minute timer is started only once the pilot tube reaches temperature.*
2. After heat shock, cool the tube rapidly in an ice bath to bring the contents to 30-35 °C. If the entire plating procedure requires more than 10 minutes, the heat-shocked tube should be placed in an ice bath for no longer than 45 minutes prior to plating.

Plating

Labeling tip: *If the Petri dishes were not labeled before sampling, the heat shock step is often a good opportunity to label. Each dish should be labeled on the bottom part of the plate (the smaller diameter piece, where the agar is poured) and not the lid (the piece with a wider diameter), because a lost lid would mean sample identification could be lost.*

1. Using a 10 mL pipette tip, aseptically (i.e., in a biological safety cabinet) pipette 2 mL aliquots into 4 10 cm Petri dishes (for 8 mL total plated volume) (alternately 4 mL into 2 dishes)
2. Pour ~20 mL of 50C TSA agar into each Petri dish and swirl to mix sample and agar.
   - *It is very important that the agar is cooled to 50 °C so that it is hot enough to damage the sample, but not so cool that it solidifies before mixing.*
3. Allow the agar to cool and solidify (agar-side down).

Incubation

Invert the sample plates (agar-side up) and place in a 32±1 °C incubator for 72 hours, with colonies counted at 24, 48, and 72 hours.

Process Controls

Each batch of agar should have a negative control plate (TSA negative plate) to ensure that the agar was properly sterilized. A water-only control can also test the sample water source. Additional controls can be added for troubleshooting or to meet laboratory quality control objectives.

Counting

Aseptic handling: *Gloves should always be used when handling Petri dishes. Lids of the Petri dish should not be removed before 72 hours to prevent cross-contamination.*

The final colony count takes place after 72 hours of incubation, but due to variability in colony morphology and growth rates, counts at 24 and 48 hours are advised. Any colonies should be recorded at each time point, and questionable spots marked for review at a later time point.

1. Examine the plates at 24 and 48 hours. If colonies visible by eye are observed, count and record data.
2. Examine and record final colony counts at 72 hours. Do not remove the Petri plate lid until after the final 72-hour count.

Clean Up

If plates are not being saved for further analysis or archiving, they can be disposed of after the 72-hour time point. All supplies that directly contact the sample solution should be sterilized by autoclave in autoclave-compatible bags. This includes test tubes, swabs, pipette tips, and plates (after 72 hours). After autoclave, the bags are cooled to room temperature and disposed of according to laboratory and institutional policies.

Wipe Method for Microbiological Sampling of Spacecraft and Environmental Surfaces

Baseline requirements for sampling a surface with a swab are described in NASA-STD-8719.27 Sections 6.5.2. and 6.6.2. A more detailed protocol is found below.

Scope and Application

This SOP is intended to be an example and does not replace any requirements found in NASA-STD-8719.27. Each laboratory should have internal SOPs as described in the laboratory quality management plan with details specific to their equipment and process.

This assay is used to sample microbial contamination on surfaces from spacecraft, parts, cleanroom/assembly areas, or ground support equipment. This assay is often referred to as the “Standard Wipe Assay Method” and is appropriate for materials that can tolerate sample collection using a damp wipe. This method is used to collect samples from surfaces between 0.1m^2 and 1m^2 per wipe. Multiple wipes may be used to sample a larger surface area.

Supplies

Sample Preparation

Consumables:
- Gloves
- 70% IPA in spray bottle
- Sterile cleanroom wipes (non-sealed edge are recommended unless required by project)
- Marker
- Sterile 50 mL centrifuge tubes
- 50 mL serological pipets
- Sterile water
- Sterile 70% IPA wipes
- Cleanroom bags and tape

Tools/equipment:
- Biological safety cabinet
- Repeater pipette
- Tube rack
- Transport container

Sample Processing and Analysis

Consumables:
- Sterile Petri dishes
- TSA agar
- Sterile rinse solution
- Markers
- Gloves
- 25 mL pipet tips
- 1000 ul tips
- 70% IPA in spray bottle
- Kim wipes
- Spore positive control
- Ice water bath
- Digital thermometers
- Timer
- Autoclave bags
- Autoclave tape

Tools/equipment:
- Tube rack
- p1000 and p10 pipettors
- 100 mL beaker
- BSC
- Vortexer
- 80 °C water bath
- 50 °C water bath
- Incubator
- Autoclave

Sample Kit and Laboratory Preparation

Notes on aseptic technique: *Preparation of sampling supplies should be conducted using standard laboratory PPE and gloves. Gloves should be wiped with 70% IPA before working in the flow bench, and anything entering the flow bench should also be wiped before it is placed on the bench.*

When working on a HEPA-filtered flow bench or BSC, pay attention to the flow direction of HEPA-filtered air. User should avoid passing hands or tools between the airflow and open sterile containers to maintain an aseptic workspace.

Materials used in sampling kits should be compatible with cleanroom requirements [for example, only using cleanroom paper, avoiding silicone adhesives, or meeting Electrostatic Discharge (ESD) requirements]. Specific compatibilities should be confirmed for each project/cleanroom. Exterior surfaces of sample kits (exterior of test tube, test tube racks, markers, etc.) should be cleaned to meet cleanroom requirements where the samples are acquired.

Day Before or Day of Sampling

1. Spray flow bench/BSC work surface with 70% IPA and wipe to clean.
2. Any supply or tool going into the BSC is to be wiped with 70% IPA.
3. Determine how many wipes you will prepare and label the 50 mL conical with your initials and date. Arrange in a tube rack.
4. Inside the BSC, place roll wipes, either in thirds or quarters so that they will fit in the 50 mL conical.
5. Using the repeater pipette or the 50 mL serologic pipette, add 20 mL of sterile water into the conical.
6. Close each conical tube, but keep the top loose for autoclaving and put through a regular autoclave cycle.
   - Place one of the conical tubes with wipe inserted generally central in the rack. Insert a biological indicator to verify autoclave efficiency.
   - Place autoclave tape on the test tube rack as a visual indicator of the whole rack.
7. After the autoclave temperature is back to ambient, move the test tubes from the autoclave to the BSC to cool. Once the tubes are at room temperature, twist the lids of the conicals so they are tight and secure.
   - Alternately, cool in the autoclave overnight, then close the lids.
8. Put each rack of test tubes in a cleanroom bag, tape shut, and store in refrigerator until ready for use.
9. Prepare sterile rinse solution according to NASA-STD-8719.27.

Day of Sampling

1. Prepare ultrasonic water bath.
2. Fill water bath with 0.02 percent volume-to-volume of polyoxyethylene sorbitan monooleate to water.
3. Set the water bath to degas for 5 minutes.
4. Turn on 50 °C water bath.
5. If samples will be heat shocked, turn on hot water bath. It takes ~2 hours to warm up to 80 °C.
6. Prepare sufficient tryptic soy agar (TSA) according to manufacturer's direction and leave to cool in 50 °C water bath. Each wipe sample is plated onto 13 plates, and each plate uses ~20 mL of agar. When autoclaving bottles of TSA, caps should be slightly loose to prevent vacuum.
7. Ensure that the incubator is on and set to 32 °C. Incubators can be cleaned by wiping with 70% IPA as necessary.
8. Sample kits and samples can be transported in an insulated transport cooler with ice packs. Ice packs may not be needed if the sample is returned to the laboratory for processing within an hour of sampling.

Sampling

Notes on aseptic technique: *Sterile gloves are recommended during wipe samples because the wipes have to be handled directly with gloved hands. Sterile gloves should be donned carefully following manufacturer's instructions. Once sterile gloves are on, care should be taken not to touch any surfaces other than the wipe and the sampled surface to avoid transfer of contamination. Mishandling wipes could result in false positives or inaccurate counts. Two operators should work together to maintain aseptic handling and avoid contact transfer. One member of the team will be the non-sterile operator, who will handle the sample tubes, sample bottles, and take notes on sample locations. The other member of the team will use a new set of sterile gloves for each sample and will receive the sterile wipe out of the test tube, perform the sampling, then return the wipe to the sample bottle, which is opened and closed by the non-sterile operator.*

Wipe Sample Collection

1. Sterile pre-moistened wipes can be used to sample between 0.1m^2 and 1m^2 per sample.
2. Non-sterile operator will carefully open test tube containing wipe and pour wipe into the waiting hands of sterile gloved sampler.
3. Wipe should be folded in half twice (1/8 of total surface area showing).
4. Measured surface area is sampled with the exposed surface of the wipe in one direction.
5. Flip folded wipe over, then refold so that the surfaces used to sample are now on the interior.
6. Rotate wiping direction 90° after each complete sampling (total of three directions of wiping).
7. Roll contaminated wipe and put into sterile 500 mL glass jar and seal jar. Sample jar is opened and closed by non-sterile operator.
8. Record sample location and total surface area sampled.

Sampling Controls

For each six or fewer samples collected, also collect a "field blank" negative control. Remove the pre-moistened sterile wipe from its tube; interact with the wipe similar to handling and folding a sample wipe. Wave the wipe through the air to simulate sampling a surface and insert into a dry, sterile, glass jar.

In the lab, create a positive control by applying a known amount of spore-positive control into jar with a sterile wipe. Process and analyze controls alongside the rest of the samples.

Transport and Storage

Transport samples to the laboratory. Samples can be stored at room temperature if processed within one hour, or stored at 4-8 °C and processed within 24 hours. Time of sampling and time of processing should be noted with sample records. A portable logging thermometer may be used to monitor sample temperature during longer transport.

Sample Processing

Notes on aseptic technique: *The samples should never be opened outside of an aseptic ISO 5 environment, which usually means inside a laminar flow bench or BSC. Some processing steps such as heat shock likely take place outside of an ISO 5 environment, so for these steps, the samples should be tightly closed. Tools, sample containers, and gloves should be wiped with 70% IPA before entering the ISO 5 environment.*

When working on a HEPA-filtered flow bench or BSC, pay attention to the flow direction of HEPA-filtered air. User should avoid passing hands or tools between the airflow and open sterile containers to maintain an aseptic workspace.

Extraction

1. In the BSC, aseptically add 200 mL of sterile rinse solution to each wipe jar and close tightly.
2. Shake the wipe jar for 5-10 seconds to unfold and agitate the wipe. Change the direction of the shaking periodically to maximize agitation. (Shake from side or side or swirl rather than up and down to avoid any leaks from the lid.) Vortexing is also an accepted approach to this step.
3. Place each jar and wipe in an ultrasonic bath, making sure the liquid level in the bath is above the fluid level in the jars. Sonicate for 120±5 seconds.
   a. Use hearing protection when the ultrasonic bath is running.

Heat Shock (Skip heat shock for vegetative samples)

The heat shock step brings the sample to 80 +/-2 °C and holds it there for 15 minutes. This process kills most vegetative microorganisms and selects for the hardy, heat-resistant microorganisms (mostly spores) that may also be resistant to other cleaning procedures and the extreme environments of space. Heat shock for 200 mL of liquid in glass bottles can take some time for the liquid to reach 80±2 °C, so it is important to have a pilot bottle to monitor temperature.

1. Place the bottle containing the agitated and sonicated wipe in a water bath at 80±2 °C for 15 minutes, as determined by a pilot bottle containing a thermometer. Make sure the liquid level in the water bath is above the fluid level in the jar.
2. After heat shock, cool the bottle rapidly to bring the contents to 30-35 °C. If the entire plating procedure requires more than 10 minutes, the heat shocked samples are placed in an ice bath for no longer than 45 minutes prior to plating.

Plating

Labeling tip: *If the Petri dishes were not labeled before sampling, the heat shock step is often a good opportunity to label. Each dish should be labeled on the bottom part of the plate (the smaller diameter piece, where the agar is poured) and not the lid (the piece with a wider diameter), because a lost lid would mean sample identification could be lost.*

1. Gently agitate the bottle just before plating to resuspend any settled particles.
2. Using a 10 or 25 mL pipette tip, aseptically (i.e., in a BSC) pipette 4 mL aliquots into 13 10 cm Petri dishes (2 mL into the last dish) for 50 mL total plated volume.
3. Pour ~20 mL of 50C TSA agar into each Petri dish and swirl to mix sample and agar.
   - It is very important that the agar is cooled to 50 °C so that it is hot enough to damage the sample, but not so cool that it solidifies before mixing.
4. Allow the agar to cool and solidify (agar-side down).

Incubation

Invert the sample plates (agar-side up) and place in a 32±1 °C incubator for 72 hours, with colonies counted at 24, 48, and 72 hours.

Process Controls

Each batch of agar should have a negative control plate (TSA negative plate) to ensure that the agar was properly sterilized. A water-only control can also test the sample water source. Additional controls can be added for troubleshooting or to meet laboratory quality control objectives.

Counting

Aseptic handling: *Gloves should always be used when handling Petri dishes. Lids of the Petri dish should not be removed before 72 hours to prevent cross-contamination. The final colony count takes place after 72 hours of incubation, but due to variability in colony morphology and growth rates, counts at 24 and 48 hours are advised.*

1. Examine the plates at 24 and 48 hours. If colonies visible by eye are observed, count and record data.
2. Examine and record final colony counts at 72 hours. Do not remove the Petri plate covers until the final 72-hour count is made.

Clean Up

If plates are not being saved for further analysis or archiving, they can be disposed of after the 72-hour time point. All supplies that directly contact the sample's solution should be sterilized by autoclave in autoclave-compatible bags. This includes wipes, pipette tips, and plates (after 72 hours). After autoclave, the bags are cooled to room temperature and disposed of according to laboratory and institutional policies.

Arithmetic Approach To Surface Bioburden Density

Factors such as surface area sampled, pour fraction, and method efficiency all need to be considered when calculating the bioburden density of a sampled surface. Bioburden density is usually expressed in CFU/m^2. Assay data and bioburden density should be documented and used to populate the mission PPEL.

A common calculation for determining bioburden density of a surface is found below. This calculation combines both swab and wipe samples. PP practitioners are considering implementation of other approaches, including Bayesian statistics.

Equation 1. Total Effective Area Sampled

$$A_s = \sum_{i=1}^{n_s} a_{si}\, f_{si}\, e_{si} + \sum_{j=1}^{n_w} a_{wj}\, f_{wj}\, e_{wj}$$

Equation 2. Total Number of Spores

$$N_{tot} = \sum_{i=1}^{n_s} N_{si} + \sum_{j=1}^{n_w} N_{wj}$$

Equation 3. Bioburden Density

$$B = N_{tot}/A_s$$

Where:

n_s, n_w	the total number of swabs or wipes, respectively, used in one group of assays
n_{tot}	the total number of samples (swabs + wipes) used in the group of assays ($n_{tot} = n_s + n_w$)
a_{si} (m^2)	the actual area sampled by the ith swab; as is typically 0.0025 m^2
a_{wj} (m^2)	is the actual area sampled by the jth wipe, which varies from wipe to wipe from 0.1 to 1.0 m^2
f_s, f_w	the pour fractions for swabs and wipes, respectively
e_s, e_w	the recovery efficiencies for swabs and wipes, respectively
A_S (m^2)	the total effective area; i.e., the total area assayed in a group corrected for the pour fraction
N_{si}	the number of spores [colony forming units (CFU)] counted in the ith swab sample
N_{wj}	the number of spores (CFU) counted in the jth wipe sample
N_{tot}	the total number of spores (CFU) counted in the whole group
B (CFU/m^2)	the bioburden density is the total number of spores divided by the total effective area sampled